THE EFFECTS OF PLUME PROPERTY VARIATION ON ODOR PLUME NAVIGATION IN TURBULENT BOUNDARY LAYER FLOWS

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To my husband, Joe Page, who has been on this journey with me
from the first *quadrilobata* to the last MATLAB

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

3DLIF	Three-dimensional laser induced fluorescence
A	Constant in logarithmic velocity law (= 1/ κ)
α	Angle between two vectors
ADV	Acoustic Doppler velocimeter
ANOVA	Analysis of variance
A_T	Total crab acceleration
$AvgC_{Amax}$	Average maximum concentration within an antennule measurement zone over an entire trial
A_x	Longitudinal crab acceleration
A_y	Transverse crab acceleration
C	Average concentration
c	Instantaneous concentration
$\bar{c}$	Time-averaged concentration
C_0	Source concentration
C_{Aavg}	Non-zero average concentration within an antennule measurement zone
C_{Amax}	Maximum concentration within an antennule measurement zone
CCD	Charge-coupled device
C_{Lavg}	Non-zero average concentration within a leg measurement zone
C_{Lmax}	Maximum concentration within a leg measurement zone
CMOS	Complementary metal-oxide semiconductor
COM	Center of mass
C_{Plume}	Mean plume concentration

C_{th}	Concentration threshold
d	Distance from the centerline
δ	Length scale for developed boundary layer
d_{50}	Average grain diameter of substrate material
ε	Turbulent kinetic energy dissipation rate/eddy diffusivity
γ	Intermittency of scalar concentration
H	Average flow depth
h	Transverse coordinate of the instantaneous concentration within a given volume
H_a	Height of the crab's antennules
H_b	Height of the concentration centroid measurement zone
κ	von Karman constant for log-law boundary layer
k_s	Effective sand grain roughness
L	Transverse integral length scale
LED	Light-emitting diode
LIF	Laser induced fluorescent
υ	Kinematic viscosity
NGDR	Net-to-gross displacement ratio
ω	Wake function
ORN	Olfactory receptor neuron
Π	Wake parameter
ψ	Crab inclination with respect to mean flow vector
$\mathbf{R}$	Source vector
$\mathbf{r}$	Crab displacement vector
Re	Reynolds number
σ	Standard deviation of transverse average concentration profile

t	Time
τ	Auto correlation decay time scale for crab movements
τ_D	Time advance used in outer chemosensor sampling technique
U	Mean flow velocity
U^+	Roughness function
$\bar{u}$	Normalized mean flow velocity
u^*	Shear velocity
$U\tau_D$	Advection distance
V_{rel}	Relative velocity of the crab with respect to mean flow
V_T	Total crab velocity
V_x	Streamwise crab velocity
V_y	Transverse crab velocity
w	Width of concentration centroid measurement zone
x	Longitudinal coordinate
X_a	Longitudinal distance from the crab to the center of the sampling zone
X_{crab}	Longitudinal coordinate of the center of the crab
y	Transverse coordinate
Y_{crab}	Transverse coordinate of the center of the crab
z	Vertical coordinate

SUMMARY

A significant body of research has focused on tracking behaviors of predators responding to prey odor plumes, yet little is known about the specific mechanisms by which predators make decisions during tracking that lead them to a source. This dissertation advances the current knowledge of plume tracking behavior by examining blue crab tracking behavior over a large range of bed-roughnesses (thereby manipulating ambient levels of turbulence), and interpreting these results with respect to chemical signal structure derived from separate examinations of plume characteristics as determined by planar laser induced fluorescence (PLIF). Foraging success and the speed of blue crabs attempting to locate the odorant source both decline consistently with increasing bed roughness. In contrast, steering (path linearity) appears unaffected by bed roughness induced turbulence. The spatial arrangement of blue crab chemosensors combined with the three-dimensional structure of odorant plumes accounts for the differential effects of turbulence on the speed and success of crab tracking behavior.

Separate examinations of tracking behavior and plume properties cannot directly examine hypotheses concerning the utility of specific chemical signal properties. In order to make a direct link between cue and behavior, three-dimensional laser induced fluorescence (3DLIF) was used to analyze three-dimensional plume structure and concentration of odor filaments that reach blue crab sensory structures. The corresponding tracking behavior was simultaneously recorded and then analyzed with a motion analysis system. These data provide the most comprehensive examination of odor signal input-behavioral output functions for animals in turbulent plumes. Crabs do

not react differentially in response to the absolute concentration of antennule spikes above threshold at their antennules but do show a state-dependent acceleration response to antennule spikes. Signals arriving at the leg sensors of blue crabs help mediate upstream motion and signal change across a single set of leg sensors is sufficient to induce turning during upstream motion. Blue crabs decrease the height of their antennules in correspondence with changing plume properties as they approach the source and the timing of signals arriving at the antennules appears to affect upstream motion. The results of this study have broader implications for the development of better tracking algorithms for autonomous robots, understanding how chemosensory signals are processed in the nervous system, and how chemosensory mediated processes can have large scale, ecological effects.

CHAPTER 1

INTRODUCTION

The goal of this study is to examine chemically mediated tracking behavior in aquatic environments. I broadly aim to determine the importance of prevailing physical flow conditions that affect signaling and examine how blue crabs, as model generalist predators, use these signals to find prey in complex environments. Specifically, I combined three-dimensional sampling of odor plume structure with simultaneous quantification of tracking behavior to determine odor plume properties that mediate successful olfactory navigation within a plume. Studying predator tracking behavior in aquatic environments will provide insights into the tracking behavior of other aquatic and terrestrial organisms. The findings from this study will have broader applications assisting the development of autonomous tracking vehicles, shedding light on nervous system organization, and providing new information on the driving forces affecting predator-prey interactions.

1.1 Background

Goal oriented search is a primary task and a selective pressure common to many organisms, which has resulted in both conspecifcs and heterospecifics evolving numerous mechanisms for detecting each other. Chemosensory information in particular mediates searches for food (Salierno *et al.* 2003), mates (Mafra-Neto and Cardé 1995), or habitat (Tamburri *et al.* 1996), avoidance responses (either morphological or behavioral) to predators (Chivers and Smith 1993; Mathis and Smith 1993), and social interactions such as establishment of dominance (Zulandt Schneider *et al.* 2000) or recognition of kin

(Booth and Katz 2000). The olfactory system is able to perform these odor recognition and association tasks both innately (Simpson and White 1990; Tabuchi *et al.* 1991; Matsumoto and Mizunami 2000) and through learning (von Frisch 1967). These interactions have the ability to structure communities by affecting organism abundance and distribution and ultimately altering community composition (Hooper *et al.* 2005). Although much is known about chemically mediated deterrence (reviewed in Hay 1996), far less is known about chemically mediated prey perception.

1.1.1 Scale of chemosensory related processes

In each case of distance chemical attraction (or repulsion), chemical compounds are disseminated by organisms into the environment either through active or passive means and are subjected to prevalent fluid forces. The dominant physical processes that determine the progression of chemical signals within the environment are size-scale dependent (Webster and Weissburg 2009). Microorganisms, such as algal gametes or bacteria, are smaller than the smallest scales of turbulence and therefore experience a completely laminar environment (*i.e.,* uniform flow, $Re < \sim 2000$), dominated by molecular diffusion, where chemical signals are present as concentration gradients (*e.g.*, bacteria [Lanning *et al.* 2008] or copepods [Moore *et al.* 1999; Jiang and Osborn 2004]). These very specific hydrodynamic conditions permit a set of chemotactic search strategies that would be impossible on larger scales where turbulent mixing breaks down predictable chemical gradients. For example, male gametes of the alga *Ectocarpus siliculosus* take advantage of chemical gradients by using chemo-thigmoklinokinesis (Maier and Muller 1986). In this strategy, the female pheromone (chemo-) modulates the chemosensory search behavior (-kinesis) of the male gametes to enhance the chances of a

successful search. Specifically, the chemical concentration causes the male gametes to maintain contact with any surface they encounter (thigmo-) and reorient when they sense a decreasing chemical gradient (klino-). This process results in eventual movement in the up-gradient direction.

As hydrodynamic conditions transition from laminar to turbulent (*i.e.*, chaotic and unpredictable flow), organisms, such as decapod crustaceans or flying insects, rely on the filamentous nature of chemical plumes to find odor sources (Vickers 2000; Weissburg 2000). Turbulence has dramatic effects on the structure of chemical plumes, causing them to break into filamentous packets of highly concentrated odorant interspersed with packets of low or no concentration (Rahman and Webster 2005), both of which are greatly variable over space and time (Justus *et al.* 2002; Liao and Cowen 2002). The spatial and temporal aspects that make up the fine-scale structure of chemical plumes provide information for olfactory-mediated behaviors (Moore *et al.* 1989; Mafra-Neto and Cardé 1995a; Finelli *et al.* 1999; Moore *et al.* 2000) and changes in this structure influence how organisms perceive and respond to chemical signals in their environment (Roelofs 1995; Cardé and Willis 2008).

1.1.2 Effect of turbulent plume properties on tracking

The location of odor sources in the habitat is one factor affecting the spatial structure of turbulent chemical plumes. In particular, the release height above the benthos and spatial separation of odor sources substantially affect turbulent plume structure. Webster and Weissburg (2001) demonstrated the considerable differences in plume structure as an effect of releasing an odor source ~2-100 mm off the benthos. The plumes released closer to the benthos experience much greater velocity shear and

turbulence intensity and therefore experience intense turbulent mixing and homogenization. Bourgoin *et al.* (2006) empirically confirmed Batchelor's hypothesis (1950) that the initial separation of pairs of plume sources will affect their subsequent dispersal. This has implications for a range of organisms that must find the source of a particular odor plume amongst a variety of attractive and repulsive plume sources in their natural environments. Experiments have already examined the importance of the spatial location of plume sources on the tracking dynamics of crayfish. Wolf *et al.* (2004) divided a single chemical source between two separated sources and observed an increase in speed and turning in tracking crayfish though there was no change in the concentration of odor present. Crayfish also spent less time in refuges and had greater tracking success with an increase in spatial complexity of chemical sources (Keller *et al.* 2001).

Turbulent mixing differentially affects the tracking performance across various groups of decapod crustaceans. When substrate induced turbulence is increased by altering bed roughness, crayfish experience enhanced tracking performance (*e.g.,* improved speed of source location, less time stopped), which is not a factor of the substrate itself (Moore and Grills 1999). Conversely, tracking blue crabs are also affected by local flow velocity but experience decreased tracking performance under more turbulent conditions (Weissburg and Zimmer-Faust 1993).

Intermittency, which is affected by the turbulent mixing created by lower release height and increased substrate roughness, appears to play a large role in the success of chemosensory searches in insects and decapod crustaceans. Greater turbulence intensity increases the cross-stream expanse of a plume and reduces the frequency of odor bursts by homogenizing the plume structure (Rahman and Webster 2005). Blue crabs in these

conditions of decreased intermittency display reduced tracking performance and increased predatory search time and effort, as well as a reduced likelihood of odor source location (Weissburg and Zimmer-Faust 1993; Jackson *et al.* 2007). Similarly, moths experience increased tracking performance with increased intermittency as measured by shorter/straighter and faster searches (Mafra-Neto and Cardé 1995).

The rate of release of a chemical signal (flux) is another major factor in the temporal distribution of the signal in turbulent chemical plumes. Keller and Weissburg (2004) found that the tracking performance of blue crabs (*e.g.*, success, speed, and directness of search) declined as the interval between odor pulses (*i.e.,* 2.5 s to 4 s interval) was increased and the length of chemical odor pulses was correspondingly shortened (*i.e.,* 2.5 s to 1 s pulses). The rate of release of an odorant is one factor that affects the intermittency of a plume. Moths of the species *Cadra cautella* experienced more direct search paths when there was a reduced interval between stimulus pulses (Justus *et al.* 2002) causing greater intermittency. Mafra-Neto and Cardé (1998) similarly found that the rate of encounter of filaments by male *C. cautella* as determined by release rate and wind speed directly affected the ground speeds and angles while tracking.

1.1.3 Search strategies

Organisms have developed a wide variety of chemosensory mediated navigation strategies as a result of evolutionary pressure to locate critical odor sources (*e.g.*, food or mates). Microorganisms inhabit very low Reynolds number environments that essentially experience no bulk flow and therefore these chemical environments are dominated by diffusion (Purcell 1977). The process of diffusion sets up chemical gradients that organisms can sense and follow by chemotaxis or chemokinesis using a variety of different strategies. Taxis generally refers to changes in heading that are determined

directly by odor field properties whereas kinesis indicates random changes when animals perceive they are moving away from the stimulus source (Dusenbery 1992). Some bacteria use indirect guidance strategies such as the 'run-and-tumble' method summarized by Berg and Anderson (1973). Using this strategy, bacteria surge ahead in a 'run' when they rotate their flagella in a counterclockwise fashion, and these bursts increase in length in response to increasing concentration. As flagella rotate in a clockwise direction, flagellar filaments flay outwards and the bacterial cell makes an abrupt change in direction as it is thrown into a 'tumble'. The increased time spent moving towards areas of higher concentration eventually results in source localization. Other bacterial species use similar indirect guidance strategies, and simply increase their speed in response to increasing concentration of an attractant, or 'run-and-turn' by continuing to rotate their flagella in a counter-clockwise direction but slowing some of its filaments when it encounters decreasing concentration (Aizawa *et al.* 2000).

Terrestrial organisms that follow surface-bound trails in two-dimensions must take several, sequential samples of an odor trail to determine the direction of increasing concentration. Dogs can correctly determine the direction of a trail of 20-minute-old footsteps within 3-5 seconds after encountering it and sniffing only five footsteps to provide them with a gradient of olfactory decay (Thesen *et al.* 1993; Hepper and Wells 2005). Ants entering midway along a trail leading from a nest to a food source cannot initially tell which direction is which but can do so after walking for a short distance on the trail (Brun 1914), possibly by using an odor gradient existing along the trail with the highest concentration near the nest (Bossert and Wilson 1963).

Terrestrial organisms following airborne odor plumes are faced with a search problem complicated by a third-dimension and the temporal and spatial fluctuations caused by environmental turbulence. Moths approach this problem of finding the source of pheromone plumes by a process of optomotor anemotaxis, whereby orientation to the wind by visual flow-field cues (*i.e.*, the relative movement of ground objects) is triggered by sensation of an attractive chemical (Mafra-Neto and Cardé 1995). Moths also utilize an internal, self-generated counterturning program that is activated by loss of contact with a chemical plume (Kennedy 1983). The combined result of these behaviors is a zigzagging (casting), upwind flight that allows the animal to follow the odor plume to its source. Field experiments demonstrate that if moths consistently flew straight upwind without counterturning, it is likely that they would be directed out of the plume due to wind shifts (David *et al.* 1982; 1983). Consequently, this pre-programmed casting behavior is crucial to their successful location of a plume source and may increase tracking efficiency by allowing the moth to continually inspect the plume for changes in odor concentration (Bell *et al.* 1995).

1.1.4 Aquatic chemosensory navigation

A wide variety of aquatic organisms have well-developed navigational strategies to follow an odor plume to its source. Chemical cues are particularly important in the aquatic environment as visual cues are virtually nonexistent at night and in deep water and greatly diminished for other organisms by turbidity or poorly developed visual systems (Wisenden 2000; Breithaupt and Eger 2002). Auditory cues are often ineffective or inefficient tracking aids as directional information is hard to discern in aquatic environments (Burks and Lodge 2002). Furthermore, chemical cues in the aquatic

environment are easily transmitted and have potential for high signal-to-noise ratios, increasing an animal's ability to discern particular chemicals above background (Wisenden 2000). Thus, aquatic organisms are excellent subjects to study the mechanisms behind tracking turbulent chemical plumes.

Weissburg (2000) suggested that there are four general ways by which organisms can utilize odor information to track odor plumes in fluid environments based on their ability (*i.e.*, low or high) to spatially and temporally resolve odor information. Organisms that have a widely distributed array of sensors on their body have the ability for high spatial resolution, therefore allowing them to compare sensors from spatially separated points in the plume while they track. This permits them to determine the direction of highest chemical concentration by comparison of signal intensity across the array. Conversely, organisms that are unable to compare information from multiple points across their body have low spatial resolution. Slow moving organisms possess high temporal resolution as they are able to take more samples of the plume while navigating. This ability to temporally average odor signals allows these animals to construct chemical gradients from otherwise filamentous turbulent plumes and follow these gradients to an odor source. Organisms that have low temporal resolution are those that move rapidly through a plume, limiting the number of odor signal samples they can collect to determine the average concentration of the plume.

Combinations of spatial and temporal abilities (*i.e.*, low or high) make up the four proposed methods that organisms utilize to resolve the information present in odor plumes and successfully track them to their source (*i.e.*, low spatial-low temporal, low spatial-high temporal, high spatial-low temporal, high spatial-high temporal). Starfish

are a good example of an organism that has the potential to exhibit both high temporal and spatial resolution. They move relatively slowly along the substrate and their radial arms are all equipped with chemosensors, giving them a wide span between their sampling points that could allow cross-body comparisons of odor. Gastropod mollusks (*e.g.*, whelks) also move very slowly, allowing them to integrate information temporally as mentioned previously. However, whelks sample the chemical information from an odor plume through their siphon, which is centrally positioned in the forward direction of movement. While this structure has some movement it is limited so the siphon essentially it acts as a point sensor (limiting spatial resolution) as the whelk moves slowly through the plume. The small size and forward antennal position of moths similarly make them poor spatial samplers while their fast flying make them poor temporal samplers as well. Male moths must move rapidly back-and-forth through a pheromone plume in order to gather enough chemical and hydrodynamic information to stay in contact with the odor and ultimately locate a mate.

A variety of marine organisms follow pheromone trails to locate mates. A series of experiments by Yen *et al.* (1998) determined that copepods employ trail-following mechanisms that are similar to ants (Brun 1914, Bossert and Wilson 1963). Males sample a female's pheromone trail repeatedly to determine her swimming direction and subsequently follow her trail with increasing speed as the concentration of pheromone increases. The male proceeds directly to the female once close enough to feel the mechanosensory disturbance caused by her swimming (Yen *et al.* 1998). Conversely, female lobsters are the ones that follow male pheromones in order to locate their mate. Pheromones are at least partially contained within the urine of male lobsters and female

lobsters show a preference for the smell of dominant males that have previously won fights with other males (Moore *et al.* 1991).

A diverse group of marine predators track the turbulent chemical plumes left behind by their prey. Many of these organisms locate the source of chemical plumes using variations of odor-gated rheotaxis, whereby the detection of an odor triggers flow oriented (*i.e.,* rheo-) motion (*i.e.,* -taxis) upstream. Like the strategies used by moths, rheotaxis relies on flow as a pointer for the general direction to the source. For example, lateral line detection of the odor and hydrodynamic signature of prey triggers dogfish (Gardiner and Atema 2007) and freshwater eels (Carton and Montgomery 2003) to swim upstream and enable them to find the source of the odor. Gastropod mollusks, such as whelks, also move upstream in response to prey odor; however, temporal integration of chemical signals, made possible by slow progression along a chemical trail, likely allows these mollusks to collapse turbulent plumes into chemical gradients (chemotaxis) that are not available to swifter moving predators in the same environments and flow conditions (Weissburg 2000; Powers and Kittinger 2002; Ferner and Weissburg 2005). Blue crabs have defined responses at the horizontal edges of odor plumes that augment their odor-gated rheotactic response. They display a side-to-side motion as they move upstream that is more pronounced when the plume width is large (Weissburg and Zimmer-Faust 1993, 1994) suggesting that blue crabs employ some mechanism of edge detection to maintain plume contact.

In a variety of sensory systems (*e.g.*, auditory, visual, and lateral line), relating stimulus properties to neural and behavioral responses has established links between sensory system organization and function (Collin and Pettigrew 1989; McKibben and

Bass 1999; Mogdans 2005). Although we understand generally that organisms are responding to properties of both ambient flow conditions and the chemical stimuli itself, it is less clear what specific properties are responsible for shaping the often complex behavior that allows organisms to follow odor plumes to their source. The constraints governing various chemosensory navigation strategies, both internal (*e.g.*, processing of odor information from the sensory organs, how the brain encodes this information) and external (*e.g.*, flow, substrate, conflicting odor information), will allow us to make similar organization-function links in chemosensory systems.

Blue crabs are an example of highly mobile predators that have little time for temporal averaging; however, their spatially separated chemosensors on the distal tips of their walking legs suggest high spatial resolution. Understanding more about chemosensory search strategies of the blue crab can tell us quite a bit about other organisms that exhibit similar search strategies. For example, odor-gated rheotaxis is common among many other highly mobile, aquatic searchers (*e.g.*, sharks, eels) allowing parallels to be made between how various organisms handle the sensory constraint of low temporal resolution. The wide spatial separation of sensors in blue crabs is potentially similar to other searchers such as starfish (even though these organisms traverse the substrate at a much slower rate) (Dale 1997; Moore and Lepper 1997) and lobsters (Devine and Atema 1982; Beglane *et al.* 1997; Grasso *et al.* 2000), thereby giving insight into general mechanisms of sensory processing based on contrasts across different body regions.

Understanding the spatial and temporal plume properties that are important for highly mobile, spatial integrators (*e.g.*, the blue crab) also gives insight into

environmental conditions where high spatial/low temporal integrators would have a chemosensory tracking advantage over low spatial/high temporal integrators. For example, blue crabs and whelks are co-occurring organisms that operate under these completely different constraints (*i.e.*, high-low vs. low-high spatial and temporal integrators, respectively). These organisms often forage for the same prey items within the same turbulent environment, giving ecosystem level ramifications to the question of which sensory modality would confer a chemosensory tracking advantage under certain ecological conditions.

1.1.5 Blue crabs as model organisms

The physical, behavioral, and environmental characteristics of large, aquatic, decapod crustaceans make them exceptional model organisms to investigate how animals encode or interpret chemical signals in turbulent plumes. Blue crabs in particular provide excellent subjects with which to examine this sensory interface between chemistry, fluid physics, and ecology as they respond to many types of chemical cues and perform stereotypical behaviors in response to these odors. Consequently, blue crabs have been frequently utilized as model organisms to study chemosensory mediated prey-tracking behavior (Weissburg and Zimmer-Faust 1994; Finelli *et al.* 2000).

Blue crabs utilize a wide array of chemosensors across their body in order to identify and locate the source of chemical stimuli. Blue crabs receive cephalic chemical stimuli from bundles of chemosensory hairs on the ends of the antennules, called aesthetascs. These structures are morphologically distinct across species of decapod crustaceans, but generally function to trap packets of water for chemo-sampling during antennule flicks. It has been demonstrated by both behavioral (Schmidt and Ache 1979;

Moore *et al.* 1991; Gleeson *et al.* 1993; Goldman and Koehl 2001; Koehl *et al.* 2001; Mead *et al.* 2003) and physical/mathematical models of flow and diffusion (Koehl 1996; Mead and Koehl 2000; Koehl 2001; Stacey *et al.* 2002; Crimaldi *et al.* 2002) that water samples are exchanged on the swift downward sweep of each antennule flick, as this is the only time that water can penetrate the dense array of hairs of the aesthetascs. The comparatively slow upward sweep of the antennules gives time for sampling and evaluation of the water packet before the next downward flick brings in a new packet for evaluation.

Thoracic chemosensors on the tips of the claws and walking legs assist in orientation during chemosensory search behavior (Keller *et al.* 2003) and function in near source search and food manipulation (Derby and Atema 1982). Previous studies have indicated that the spatial comparisons of signal intensity across widely spaced chemosensors can help orient organisms within the boundaries of chemical plumes (Weissburg and Zimmer-Faust 1994; Zimmer-Faust *et al.* 1995; Webster *et al.* 2001, Weissburg *et al.* 2002). Deafferentation experiments have suggested that the chemosensors on the distal tips of blue crab appendages may function in this manner (Keller *et al.* 2003), allowing the crab to maintain contact with the chemical plume by comparisons of concentration across the width of the body. This spatial comparison allows us to utilize blue crabs as model organisms for other trackers that require spatially integrated information for successful tracking behavior (*e.g.,* starfish).

It is ultimately the combination of the cephalic and thoracic chemosensors that help orient a blue crab within a chemical plume. Research by both Weissburg and Zimmer-Faust has indicated that blue crab tracking behavior is governed by both

upstream surges in response to concentration spikes and spatial orientation within a plume mediated by plume boundary sensation (Weissburg and Zimmer-Faust 1993; Zimmer-Faust *et al.* 1995, Weissburg *et al.* 2002). Computer simulations of blue crab tracking behavior modeled using these two guiding principles result in tracks similar to those observed across a variety of crab tracking studies (Weissburg and Dusenbery 2002). This information has led to the hypothesis that antennule sensors are responsible for the upstream portion of a track and the leg sensors are responsible for orientation during tracking (Keller *et al.* 2003). These two populations of sensors are at very different heights in the water column and, presumably, encounter very different spatial aspects of the plume (Webster and Weissburg 2001). Separation of behavioral control into two sets of sensors encountering unique plume environments provides an opportunity to dissect the control of tracking behavior into its components, thereby potentially making it easier to decipher the strategies that organisms utilize to find distant chemical attractants.

Blue crabs provide excellent subjects for studying the role of instantaneous properties of odor plumes in chemosensory search behavior, as they appear to rely on properties of individual odor filaments for information. Filaments arrive at the organism's sensors in brief bursts and, although the time averaged structure of a plume is predictable, averaged structures take very long sampling times (on the order of 200 s or more) to resolve (Webster and Weissburg 2001). Crabs and lobsters move quickly through plumes and the time period needed for adequate time-averaged sampling is too long to be practical for their use (Webster and Weissburg 2001; Weissburg *et al.* 2002); hence, these crustaceans must extract odor plume information from the filaments

themselves. Understanding what plume properties are important for successful odor plume navigation and the mechanisms by which blue crabs utilize these signals is widely applicable to other organisms that rely on filaments as they engage in tracking behavior (*e.g.,* moths and lobsters).

As discussed previously, the hydrodynamic characteristics of the environment (*e.g.*, advection, turbulence intensity), are critical determinants of the structure of chemical plumes and, consequently, the success of olfactory searches in organisms such as decapod crustaceans (Weissburg and Zimmer-Faust 1993, 1994). Greater turbulence intensity increases the cross-stream expanse of a plume and reduces the frequency of odor bursts by homogenizing the plume structure (Rahman and Webster 2005), thereby making edge determination more difficult. Blue crabs in these conditions display reduced tracking performance and increased predatory search time and effort, as well as a reduced likelihood of odor source location (Weissburg and Zimmer-Faust 1993; Jackson *et al.* 2007). Understanding exactly how particular crustaceans, like blue crabs, utilize the information in odor plumes may elucidate search strategies that may be optimal under specific conditions (*e.g.,* increased or decreased turbulence). In turn, this may allow us to predict the search strategies used by other animals in these same conditions.

Similar to observations on other sensory modalities (*e.g.*, lateral line in fish [Mogdans 2005; Bleckman *et al.* 1989]), studies on blue crab chemosensory guidance suggest specific roles for particular sensors that may depend on, or take advantage of, particular signal properties. The detection of intense odor bursts by the antennae stimulates upstream locomotion, allowing crabs to navigate closer to the source (*i.e.*, odor-gated-rheotaxis, Weissburg and Zimmer-Faust 1993; Zimmer-Faust *et al.* 1995;

Keller *et al.* 2003). Additionally, crustaceans appear to use bilateral contrast of stimulus concentration across their body to detect when they have exited a plume in a cross-stream direction (Keller *et al.* 2003), allowing crabs to determine the cross-stream location of odor sources. Flow visualization data indicates that plume structure at the height of the antennal sensors is more filamentous while signal at the height of the leg sensors is more homogeneous (Rahman and Webster 2005) suggesting that each set of sensors uses particular aspects of local signal structure to modulate very different aspects of blue crab tracking behavior. This functional separation of along-stream and cross-stream orientation into sets of sensors located in different parts of the chemical plume makes blue crabs ideal for studying how individual sensors may be tuned to take advantage of signals particular to specific microenvironments.

In addition to defined responses at the horizontal edge of an odor plume, blue crabs also adjust behavioral patterns when in close proximity to an odor source. Although time-averaged odor burst properties (*e.g.*, mean values of pulse height and onset of pulse slopes) vary predictably with distance from the source under the same fluid regime (Moore and Atema 1991; Moore et al. 1994), instantaneous properties of plumes are unpredictable. Variation in time-averaged burst properties with distance from source and altered near source tracking behaviors (*i.e.*, waving of claws and digging of walking appendages into the substrate [Page, *pers. obs.*]), suggests that blue crabs use plume properties to determine distance from the source. Lobsters also exhibit a switch to near-source tracking behavior: an initial phase (characterized by low walking speed and large heading angles) is followed by an approach where both speed and accuracy increase to a maximum. Distance search is likely governed by cephalic sensors, and is maintained until

input from the walking legs guides the final search (Devine and Atema 1982). The functional separation of orientation mechanisms into different sensors is yet another reason blue crabs are excellent organisms to determine the plume properties that are necessary to mediate particular components of tracking behavior, such as this near source switch.

Findings from studies such as Keller *et al.* (2003) suggest that three-dimensional plume structure should affect blue crab tracking; however, no studies have determined the relevant plume properties necessary for successful odor plume navigation. Previous studies have examined planar plume structure and correlated general plume properties to tracking behavior under similar flow conditions (Webster and Weissburg 2001; Weissburg *et al.* 2002; Keller *et al.* 2003; Weissburg *et al.* 2003). However, these generalizations do not determine the specific plume properties that are essential for blue crabs to determine the best track through a chemical plume. Simultaneous correlation of three dimensional plume structures with blue crab tracking behavior is necessary to determine the relevant plume properties that govern behavioral decisions during tracking.

1.2 Impacts and contributions

1.2.1 Autonomous vehicles

Understanding the chemical plume tracking behavior of organisms has great ramifications in developing autonomous tracking technology to locate the source of hazardous chemical plumes such as gas or chemical leaks or other pollution sources. Attempts to create such autonomous vehicles by incorporating animal sensing strategies occurred as early as 1993 when Sandini *et al.* worked on a terrestrial unit to follow gas concentration gradients. Sandini *et al.* took inspiration from the "swarm intelligence" of

the ant and bee colonies outlined by E. O. Wilson (1971), constructing a robot that processed odor plume signals spatially and temporally across a pair of sensors. Autonomous search came to the forefront in a 1994 symposium sponsored by IEEE on Autonomous Underwater Vehicle Technology. At this symposium, Consi *et al.* (1994) presented data on an underwater mobile robot that followed chemical concentration gradients. This robot was also equipped with two chemical sensors and was modeled after the chemosensory strategies of lobsters. In both cases, the robots were programmed to turn towards the sensor that was experiencing a higher concentration. Each robot was only occasionally successful as filamentous odor patches would often steer the robots off course and they were unable to recover the plume once they had exited its general boundaries.

A second generation of chemical plume tracking robots employed biomemetic strategies that incorporated both chemotactic and rheotactic mechanisms to track odor plumes to their source. Ishida *et al.* (1994) found that incorporating chemical and anemometric sensors (similar to moths tracking plume) on an autonomous vehicle allowed the vehicle at each step to determine the actual location of the odor source with a high probability. The time to find the source (shortest ~5 min) was greatly reduced from studies on purely chemosensory robots and experiments with either stopping or retracing steps when the odor concentration dropped below a prescribed threshold both increased the likelihood of successfully locating the odor source. Hayes *et al.* (2002) used wind sensors to enable upstream surge behavior after chemical contact (similar to moths) and a gyroscope was used as a flow detection device for Grasso and Atema's (2002) underwater robot based on a lobster, which detected the relative chemical concentration

at each of two sensors and moved the robot cross-stream towards the sensor that had experienced the highest concentration. Farrell *et al.* (2005) implemented a 'behavior based planning' strategy whereby individual behaviors, such as reacquiring a lost signal, are based on strategies in insects, and synthesized from the bottom up to create a comprehensive tracking strategy. Utilizing this approach, they were able to construct a long-range autonomous underwater vehicle that could track a plume over hundreds of meters but was only accurate within 13 meters.

Despite these attempts, applying search strategies derived from interpretation of animal navigation behavior has not allowed autonomous agents to replicate the level of success of the animal itself. As mentioned previously, navigating in turbulent chemical plumes relies on encoding plume structure in both spatial and temporal domains but little is known about specific signal properties that are important tracking. Simultaneous quantification of three dimensional odor plume structure and crab tracking behavior is necessary to more precisely determine critical plume properties used by blue crabs in navigation through chemical plumes. Identifying these properties may enable us to develop an autonomous vehicle that will be able to approach the tracking performance and success of actual organisms.

1.2.2 Nervous system organization

This thesis examines the properties of chemical plumes that are important to navigation and the behavior that results from encounter of those specific plume properties. In between these two ends, crabs must actually sense and process odor information before exhibiting particular behaviors. Any meaningful investigation into the chemical ecology of nervous system organization and processing must be approached

by integrating information from a variety of perspectives, including chemistry, ethology, neuroscience, evolution, and ecology. While it is the integration of these varied disciplines that will ultimately lead to the major advancements in the field, information garnered from any one of these disciplines stands to contribute significantly to our understanding of neural processing and provide a basis for continued study in other fields.

As discussed earlier, the dynamic (time-dependent) properties of odor plumes provide crucial information for organisms (*e.g.*, moths) tracking turbulent plumes (Willis and Baker 1984; Mafra-Neto and Cardé 1995; Justus *et al.* 2002) and seem to carry most of the information to sensory systems (Vickers *et al.* 2001). While we are aware of the importance of temporal information to chemosensory mediated tracking, the timing patterns that are necessary to produce certain navigational behaviors are not fully understood. Research to understand the dynamic properties that are detectable and important to organisms has traditionally approached the problem by examining the basic responses of sensory receptors to various stimuli. On such a small scale, it is much easier to control the presentation of the stimuli to a particular chemosensor and monitor the response. Vickers et al (2001) used this approach to demonstrate the variable response of moth olfactory neurons to different levels of dynamic stimulation. These responses have also been examined in wind tunnels through pulsed release of chemicals (Bau *et al.* 2005) and source concentration variation (Justus *et al.* 2005).

While approaching the problem of dynamic stimulation from the level of the sensor is important, the excitation of a sensory cell to particular stimuli does not give any information on the excitatory or inhibitory nature of that stimuli or its integration with

stimuli from other sensors. We must understand the effect of dynamic stimulation on the actual behavior of an animal to understand this level of sensory processing and integration. This information thus far has been generalized because we have been unable to directly measure arrival of stimuli at the level of a sensor while an animal is in the process of tracking. The ability to quantify the signals arriving at a blue crab's chemosensors simultaneously with the resulting behavior over the entire length of a search will provide crucial information about the temporal patterns of stimuli important to chemosensory tracking, and how those stimuli are integrated by the crab's nervous system to produce certain behaviors.

Blue crabs take in odor information through their aesthetascs and other chemosensory cells on the distal tips of the claws and the walking legs. These sets of sensors control different aspects of the blue crab's behavior indicating that they are processed differentially at some point within the central nervous system. The specific flow environments experienced by these sensors (Rahman and Webster 2005, Jackson *et al.* 2007) raises the possibility that the properties of these individual sensors are designed to encode specific signal types for the separate functions of forward motion and steering within a chemical plume. Examining the behavior that results from these particular stimulation patterns of these sensors may give us additional information on how crabs process multiple, and perhaps conflicting, signals. Information from the level of the sensor has already led to hypotheses about "olfactory multitasking" in the central nervous system of moths (Vickers *et al.* 2001). The similarities between the olfactory pathways of decapods and insects (Schmidt 2007), suggests that understanding of chemosensory coding and processing in aquatic crustaceans (*e.g.*, blue crabs) can be directly related to

olfactory processing in terrestrial insects (*e.g.*, moths). Simultaneously examining plume properties and the resulting blue crab tracking behavior makes a direct connection between signal properties and chemosensor function and this information will help test such hypotheses as the olfactory multitasking proposed by Vickers *et al.* (2001).

1.2.3 Ecological interactions

Chemosensation is considered a basic sense common to most organisms because chemicals can participate directly in biochemical reactions without requiring a sensory transduction step (Dusenbery 1992). Since chemical stimuli are so ubiquitous and are routinely utilized by organisms to mediate their inter- and intraspecies interactions (Dusenbery 1992; Zimmer and Butman 2000), animal responses to chemical stimuli can have significant effects on community structure and composition (Dill 1987; Raimondi et al. 2000; Turner et al. 2000; Trussell et al. 2003).

Blue crabs are highly mobile predators with foraging behaviors that have significant effects on the community structure of the estuarine environments in which they are found (Virnstein 1977). Annual fluctuations in the blue crab population in the Chesapeake Bay are directly correlated to annual fluctuations in the abundance of one of their primary prey items, *Macoma balthica* (Hines *et al.* 1990) and blue crab presence is a critical determinant of the habitat dependent (*e.g.,* sandy vs. muddy habitat, burrowing depth) persistence of prey species in the Bay (Eggleston *et al.*1992; Ebersole and Kennedy 1995; Seitz *et al.* 2001). The various bivalve prey of blue crabs (*e.g.,* oysters) are filter feeders that are particularly important in estuaries to filter out the vast amounts of suspended particulate matter thereby increasing water quality (Dame *et al.* 1984). Whether buried in the sand or creating reefs, bivalves also stabilize sediments and create

hard structure on the benthos, both of which are critical for species recruitment (Peterson *et al.* 2003). The cascading effects of blue crab predation are also commercially important as they and the species they prey on often are economically as well as ecologically important (Peterson *et al.* 2003; Kennedy and Cronin 2006). Understanding the signal properties used during orientation permits predictions of environments that enable or inhibit orientation, which in turn, may furnish predictions on patterns of blue crab predation in nature.

Findings from blue crab studies may also give us insight into the search behaviors of other predators that are important in community structuring (*e.g.*, sea stars [Menge 1976]) as well as the pheromone guided, mate-finding behavior in insects, which may themselves be highly detrimental to commercially or ecologically important plant species (*e.g.,* potato tuber moth [Coll *et al.* 2000]). Rapid movement through an odor plume is something that is common between blue crabs (*i.e.,* aquatic tracker) and moths (*i.e.,* terrestrial tracker) as they both have to encode information from highly intermittent odor filaments relatively quickly in order to find an odor source. However, blue crabs have the ability for high spatial integration whereas moths do not and each organism uses a different search strategy (*i.e.*, odor-gated rheotaxis vs. optomotor anemotaxis). Consequently, understanding the 'rules' that govern tracking in blue crabs can be used to determine what parts of each strategy may be particular to high vs. low spatial integrators and what aspects are common to low temporal integrators. Both strategies involve orientation to the prevailing flow conditions in some manner to guide their tracking behavior, providing another common mechanism between the different fluid realms.

CHAPTER 2

CONSEQUENCES OF BED ROUGHNESS EFFECTS ON BOUNDARY LAYER TURBULENCE FOR ODOR-TRACKING BEHAVIOR IN BLUE CRABS (*CALLINECTES SAPIDUS*)

Abstract

I examined tracking behavior in light of laser-induced fluorescence (LIF) measurements of odorant plume structure to investigate how turbulence affects the three-dimensional structure of odorant plumes and subsequently mediates olfactory search efficiency and success in my model organism, the blue crab (*Callinectes sapidus*). The turbulent characteristics of the saltwater flume used for behavior trials was systematically varied by changing the bed substrate roughness to create smooth, transitional, and fully rough flow conditions that mimicked those created in the LIF study. Generally, increasing bed roughness caused greater mixing, decreased the time-averaged concentration and concentration variance, and increased the plume width and homogeneity. Foraging success and the speed of blue crabs attempting to locate the odorant source both decline consistently with increasing bed roughness. The variation in signal structure at the height of the antennules among bed roughness treatments explains the observed behavior differences in crab foraging speed. In contrast, steering (path linearity) appears unaffected by bed roughness induced turbulence. The correlation function for odorant concentration at sensors separated across the width of the carapace is examined as a function of plume width and homogeneity among bed roughness

treatments, and ultimately the correlation function is related to the spatial position of tracking blue crabs. The spatial arrangement of blue crab chemosensors combined with the three-dimensional structure of odorant plumes accounts for the differential effects of turbulence on the speed and success of crab tracking behavior.

*Note: Fluids experiments in this chapter were completed as part of Shika Rahman's Ph.D. thesis (Rahman 2002). The full manuscript, Jackson *et al.* (2007), which incorporates both the behavioral and fluid mechanical components, as published in Limnology and Oceanography.

2.1 Introduction

The importance of flow and turbulence to the ecology of aquatic benthic organisms has been widely reported (*e.g.*, Nowell and Jumars 1984; Hart *et al.* 1996; Keller and Weissburg 2004), but the mechanisms linking the flow characteristics to ecological processes remain poorly quantified. Bed roughness is one environmental variable that influences turbulence and is known to be important in shaping the character of boundary layer flows in aquatic systems (Nowell and Jumars 1984). Roughness has also been of interest to engineers and atmospheric scientists for many decades and is known to influence the intensity and spatial distribution of turbulence (Jiménez 2004). Mobile benthic organisms progressing through an estuarine environment encounter a variety of substrates that introduce various levels of turbulence and consequently influence the mixing of chemicals and the structure of ambient chemical signals. Turbulence-induced changes in chemical signal structure are an important determinant of chemosensory abilities in the laboratory (Moore and Atema 1991; Weissburg and

Zimmer-Faust 1993; Ferner and Weissburg 2005) and field (Finelli *et al.* 2000; Smee and Weissburg 2006), and thus, the environmental characteristics that alter plume structure may exert significant ecological effects. The goal of the current study was to explore the consequences of the physical characteristics of the flow environment for chemical signal structure in a benthic boundary layer and animal behavior.

Chemical signaling plays an important role in aquatic systems by mediating interactions between organisms, including mate location, prey tracking, or predator identification (Weissburg 2000; Vickers 2006; Koehl 2006). The proficiency with which organisms locate odorant sources, such as prey or mates, has been shown repeatedly to be contingent on the flow environment (Weissburg and Zimmer-Faust 1993; Mafra-Neto and Cardé 1994; Belanger and Willis 1996), which clearly links the ecology of these organisms to hydrodynamic mechanisms. Navigating towards an odorant source relies on an animal's ability to encode plume signal structure conveyed by spatial and temporal patterns of chemical stimulus intensity (Moore and Atema 1991; Weissburg and Zimmer-Faust 1994; Finelli *et al.* 2000). This is a challenging task given that turbulent mixing creates a signal structure that exhibits great spatial and temporal variability (*e.g.*, Webster and Weissburg 2001; Crimaldi *et al.* 2002). From a spatial perspective, the plume consists of a highly convoluted filament surrounded by odorless fluid. From a temporal perspective, the signal erratically fluctuates as the filament structure moves past a sensor.

Many aquatic predators move quickly through plumes, strongly suggesting that the time-averaged structure is not practically useful as a source of information for them because the time period needed to acquire reliable estimates of average local concentration is too long (Webster and Weissburg 2001). Rapidly mobile benthic

consumers consequently are thought to require information present in the filament structure produced by turbulence to navigate to an odorant source (Moore and Atema 1991; Weissburg and Zimmer-Faust 1994; Webster and Weissburg 2001). Increased turbulent mixing intensifies the homogeneity of chemical plumes, thereby altering the filament structure and degrading tracking performance (*e.g.*, slower speed, increased stopping, and indirect trajectories) of blue crabs (Weissburg and Zimmer-Faust 1994). Alternatively, Moore and Grills (1999) and Ferner and Weissburg (2005) observed that the performance of crayfish and whelks, respectively, improved with increased turbulence. However, it is important to note that the range of turbulence parameters over which tracking performance has been studied is small, particularly compared to what is seen in the field.

Blue crabs (*Callinectes sapidus*) are crustacean predators with two sets of chemosensors that are responsible for regulating different aspects of the organism's tracking behavior (Keller *et al.* 2003). Chemosensors on the antennae, which are elevated on the crab's body, control the forward movement of the crab via odor-gated rheotaxis (a strategy whereby odorant arriving at these sensors is coupled with mechanosensory information to induce upstream motion towards the odorant source). Chemosensors on the crab's legs, which are spatially separated and near the benthos, are believed to mediate turning relative to the plume structure. The combination of information gathered by these two sets of sensors induces an upstream, zigzag motion that allows the crab to stay within the odorant plume until it is at a distance from the source sufficient to initiate a near-source search behavior (Weissburg and Zimmer-Faust 1993; 1994; Keller *et al.* 2003). The combination of sensors at different heights in the

water column coupled with upstream motion means that blue crabs can acquire time-varying, three-dimensional (3D) information about their environment. Prior research has relied on coupling behavior to two-dimensional (2D) information (at best), thereby creating a significant gap in our understanding of the sensory basis of tracking behavior.

Quantitative examination of plumes has demonstrated that the concentration field varies with bed roughness. Rahman and Webster (2005) noted that the distributions of the fluctuating and time-averaged concentrations in the vertical (z), transverse (y), and streamwise (x) directions are influenced by the bed roughness. Hence, I hypothesized that variation in turbulent mixing due to bed roughness would affect tracking behavior in crustacean predators by altering the signal structure at different heights in the water column and by altering the width and homogeneity of the plume.

The objective of this study is to examine the effects of environmentally induced turbulence on predatory tracking behavior and relies on examination of the effects of bed roughness on chemical plume signal structure by Rahman (2002), which quantified odor fields in an environment fluid dynamically similar to prevailing conditions in behavioral experiments. The large range of substrates encountered by naturally foraging benthic organisms motivates the need to specifically examine the connections among substrate properties, plume signal structure, and chemosensory navigation. Here, I summarize the effects of bed roughness on the chemical signal structure and examine the effects of roughness induced changes in signal structure on the prey finding ability of a generalist predator, the blue crab (*Callinectes sapidus*). I also explore the possibility that sensors on different appendages of organisms may have properties that allow them to function best under the specific environmental conditions in which they operate. Sensors that are

matched in this way to prevailing conditions of a specific stimulus have been said to be "tuned" to their sensory environment (Atema 1985; Keller *et al.* 2003). Because particular chemosensory appendages are placed at different heights in the water column, the chemical signals available to each appendage vary substantially within the same plume. Hence, I used different substrate roughness to create plumes with different vertical plume structure to determine the consequences of vertical variation of the concentration field on *C. sapidus* behavior. The results of this experiment suggest the 3D structure of plumes is an essential determinant of tracking since sensors receive height-dependent patterns of chemical stimulus intensity. As the two sets of sensor populations seem to mediate different aspects of blue crab behavior and they are located at different heights in the water column, understanding 3D plume structure will allow new insight into the differential behavioral control of these sensors.

2.2 Behavioral methods

2.2.1 Flow environment

Experiments to visualize *C. sapidus* behavior were carried out in a 12 m × 0.75 m recirculating saltwater flume (Keller *et al.* 2003). The bulk flow velocity in the channel, determined using an electromagnetic flow meter that monitored volumetric flow rate in the delivery pipe, was steady at 5 cm s^{-1}, which is within the range of in situ flow speeds encountered by *C. sapidus* (Smee and Weissburg 2006). Turbulence level was varied in separate flume trials by using four substrate materials to line the flume bed (fine sand d_{50} = 1 mm, small gravel d_{50} = 2.5 mm, pea gravel d_{50} = 11.5 mm, large gravel d_{50} = 21 mm, where d_{50} represents the average grain diameter of the substrate material), representing a

range of sediments that *C. sapidus* naturally encounters (Meise and Stehlik 2003; van Montfrans *et al.* 2003). The flow depth (H) was 0.25 m in all trials.

Rahman (2002) conducted parallel experiments in a 24.4 x 1.07 m flume to quantify the odorant plume structure. The bed substrate matched that of the behavior trials except for the smoothest bed treatment, in which fine sand was used for the behavior observations to allow the crabs to walk normally, and a smooth polyethylene sheet was used in the concentration measurement experiments. The water depth was H = 0.20 m and the bulk flow velocity was 5 cm s^{-1} in these experiments.

I verified that the flume used for behavioral observations generated similar boundary-layer characteristics as the flume used for quantifying odorant plume structure. A SonTek MicroADV was used to measure the mean flow velocity profile for each bed condition, using 5-min velocity records collected at 20 Hz. Data were collected at 36 locations above the bed; the lower 15 locations were spaced by 2-mm increments, the next 10 were spaced by 4-mm increments, and the upper 11 locations were spaced by 8-mm increments. These data were optimally fit to the rough-bed "law-of-the-wall" profile equation to determine the appropriate boundary-layer parameters using the following equations (*see* Rahman and Webster 2005 for detailed procedure):

$$\frac{u}{u^*} = \frac{1}{\kappa}\ln\left(\frac{(z+\epsilon)u^*}{\nu}\right) + A - \Delta U^+ + \frac{2\Pi}{\kappa}\omega\left(\frac{z+\epsilon}{\delta}\right)$$

$$\frac{u}{u^*} = \frac{1}{\kappa}\ln\left(\frac{(z+\epsilon)u^*}{k_s}\right) + C + \frac{2\Pi}{\kappa}\omega\left(\frac{z+\epsilon}{\delta}\right)$$

$$\Delta U^+ = \frac{1}{\kappa}\ln\left(\frac{k_s u^*}{\nu}\right) + A - C$$

Table 2.1 shows that the bed shear stress, roughness function, effective sand roughness height, and roughness Reynolds number were nearly identical for each bed

Table 2.1. Flow characteristics for the four bed roughness treatments in the two flumes used in this study, where $u*$ = bed shear velocity, ΔU^+ = roughness function, k_s = the effective sand roughness height, and roughness Reynolds number = $k_s u*/\nu$. Data corresponding to the flume for concentration measurements are from Rahman and Webster (2005).

Flow regime	d_{50} (mm)	$u*$ (mm s^{-1})	ΔU^+	k_s/d_{50}	Roughness Re
Flume for concentration measurements					
Smooth	0	3.08	0	-	0
Transitional	2.5	3.44	1.7	1.41	12
Transitional	11.5	4.35	6.6	1.29	65
Fully rough	21.0	5.82	10.8	2.88	350
Flume for behavior observations					
Smooth	1	3.01	0	1.0	3
Transitional	2.5	3.44	1.9	1.49	13
Transitional	11.5	4.49	6.3	1.10	58
Fully rough	21.0	5.92	11.0	2.99	380

treatment in the two flumes. Hence, I concluded that the flow environments and velocity characteristics in the two flumes were similar and that valid connections could be made between the data sets. The roughness Reynolds numbers indicate that the substrate manipulations created substantial changes in boundary-layer properties and produced flows that spanned hydrodynamically smooth, transitional rough, and fully rough regimes (Daily and Harleman 1966).

2.2.2 Chemical plume release

The chemical plume was created by an isokinetic release (*i.e.*, effluent velocity matched to the bulk flow) from a 4.7 mm inner diameter nozzle in both the concentration measurements and behavior trials. The nozzle center was located 25.4 mm above the top of the bed material representing odorant release from prey organisms on or near the benthos. A streamlined faring on the nozzle minimized the wake perturbation; hence,

mixing of the chemical plume resulted from the velocity shear and turbulence of the bed boundary layer.

2.2.3 Behavior trials

Blue crabs were purchased from Gulf Specimen Marine Laboratory, Panacea, Florida, USA, and collected from Wassaw Sound, Georgia, USA, and associated tributaries using commercial crab traps. Animals were housed in recirculating artificial saltwater tanks (salinity: 30-32; water temperature: 25ºC) at the Georgia Tech Environmental Fluid Mechanics Laboratory until use. Crabs were allowed to acclimate for 48 h after introduction to the holding tanks prior to initiation of behavioral assays and were used within 2 weeks of arrival. Crabs were fed small amounts of shrimp every other day for the duration of the trials to assure that they were consistently hungry (would still accept food) and were used after starvation periods ranging from 24h – 48 h.

Behavior of navigating blue crabs was visualized and recorded by a charge-coupled device (CCD) video camera mounted 2 m above the test section, with a 3.5 mm wide angle lens such that the image encompassed a test section 1.5 m long by 0.75 m wide. Individual crabs were outfitted with a backpack with two light-emitting diodes (LEDs) mounted along the widest axis on the crab carapace (Weissburg and Dusenbery 2002). The flume area was darkened to minimize visual distraction to the crabs and increase the contrast between the backpack lights and the background light level. Crabs were held in a cage at the downstream end of the test arena (1.5 m from the odorant source) for a 10-minute acclimation period prior to their release into the test section of the flume. Odorant plume release was initiated prior to the introduction of crabs into the flume to ensure that a developed odorant plume was present for the entirety of the

acclimation and trial periods and that a potential forager contacted the plume. Once the cage door was raised, crabs were given 10 minutes to initiate tracking by exiting the cage and an additional 10 minutes to complete their motion across the test section. Within this time period, crabs either moved across the test section and missed or found the odorant source, or remained stationary for virtually the entire time; no animals were in the process of tracking at the end of the test period. Crabs that did not leave the cage or immediately went backward beyond the start line after exiting the cage were not included in the study data. Crabs were only used once in these experiments.

The positions of the two LEDs were recorded onto videotape and analyzed using a motion analysis system (Motion Analysis Corp., model VP110) over the entire length of the test section (1.5 m). Raw data were acquired at 30 Hz during digitization, but path kinematics were calculated with data downsampled at a final rate of 5 Hz. The system digitized the coordinates of the animal on a frame by frame basis, allowing calculations of linear and angular velocity, acceleration, turning rate, path linearity (net to gross displacement ratio or NGDR, ranging from 0 to 1, where 1 indicates a completely straight path from origin to destination), and body angle.

Stimulus solutions were made in high and low concentrations by soaking intact, previously frozen shrimp (7.0 g and 2.21 g, respectively) in 1 L of seawater for 1 h immediately prior to experiments. Control experiments were conducted with plain seawater released from the delivery system and were used to examine the effects of substrate *per se* on locomotion behavior.

2.2.4 Analysis

Percent tracking success rate was calculated by comparing the number of animals that contacted the odorant source (Hit) to the total number of attempts (Hit + Miss). To account for motivational state of the animals, a Miss was included in the data set only if the animal consumed a piece of shrimp after its unsuccessful tracking attempt. Data were analyzed using chi-square contingency table analysis to compare success rates across turbulence (substrate size) and stimulus concentration treatments.

Efficiency of navigation towards the odorant source was analyzed by examining path kinematics of crabs successfully tracking to the source (*i.e.*, speed and net to gross displacement ratio). I performed an initial analysis of kinematic performance in order to examine the effects of substrate on movement in the absence of odorant stimulation. This analysis consisted of data from animals that lacked odor stimulation or that did not track to the source when challenged with odorant, since previous studies (*e.g.*, Weissburg and Zimmer-Faust 1994; Keller *et al.* 2003) have suggested similar behaviors between these two groups. I nonetheless used odorant treatment level (0, Low, High) and substrate as factors in a 2-way analysis of variance (ANOVA) design. This initial analysis suggested that the kinematic performance of searching animals is not explained by the substrate treatment *per se* (*see* Section 2.3). Consequently, a 2-way ANOVA with post-hoc Tukey-Kramer analysis was used to determine the effects of bed roughness and stimulus concentration (Low, High) on navigational performance (*i.e.*, speed and NGDR) of successful trackers. I also used a 3-way ANOVA, with substrate, odor concentration, and downstream distance as model effects. Distance corresponded to 3 levels equaling 0-50, 51-100, and 101-150 cm downstream. Note that I explicitly included substrate as a main

effect in the analysis of both tracking and non-tracking individuals even though I was unable to randomize substrate treatments across trials. Randomization of substrate treatments was limited due to the logistical difficulty of replacing the entire bed with different material between trials. However, trials on different substrates took place over multiple periods, and I interspersed no odorant controls with trials involving odorant, so that each treatment was tested with animals collected over several different seasons. Approximately 10% of the crabs either failed to consume shrimp after an unsuccessful tracking event or did not track during the 10 min period, with no detectable differences in animal responsiveness across treatments.

2.3 Behavioral results

Contingency table analysis demonstrated that percent tracking success of crabs varied significantly across the test conditions (Table 2.2; $G = 28.7,\ df = 10,\ p < 0.001$). Control crabs never navigated to the stimulus nozzle regardless of bed conditions. However, increasing bed roughness and decreased source concentration both

Table 2.2. Percent success for the behavior trials. Control indicates the number of crabs tested in absence of odorant stimulation for each bed-roughness treatment. Cross treatment discrepancies in trial numbers reflect differential success rates across treatments, which required some treatments to be tested more thoroughly to acquire data that could be statistically analyzed.

Stimulus	Low odorant concentration				High odorant concentration			
Substrate	Sand	d_{50} = 2.5 mm	d_{50} = 11.5 mm	d_{50} = 21 mm	Sand	d_{50} = 2.5 mm	d_{50} = 11.5 mm	d_{50} = 21 mm
Control	14	28	28	30	-	-	-	-
Miss	12	10	38	15	6	6	26	18
Hit	8	4	13	2	11	11	32	8
Percent success	40%	29%	25%	12%	65%	65%	55%	31%

diminished tracking success for animals exposed to odorant ($G = 8.3,\ df = 3,\ p < 0.05$; $G = 15.5,\ df = 1,\ p < 0.05$ for bed roughness and concentration, respectively), and there was a strong indication of a substrate-concentration interaction ($G = 11.28,\ df = 6,\ p = 0.082$). The source of this interaction appears to have been related to the observation that tracking success was less affected by substrate roughness when source concentration is high. Crabs tracking the more concentrated source were only minimally affected over the first two roughness conditions, whereas tracking success declined greatly over these two treatments for crabs exposed to the dilute stimulus. Test statistics of this analysis and the subsequent analysis of kinematics are presented in Table 2.3.

The effects of substrate *per se* on locomotion were not consistent with changes in behavior during odor tracking (*see* below), strongly suggesting that the changes I observed during odor tracking were specific responses to the odorant environment. Analysis of the kinematic performance suggested that animals in the no-odorant controls performed similarly to animals not tracking to the source (Figure 2.1), which is consistent with numerous other studies (*e.g.*, Weissburg and Zimmer-Faust 1994; Keller *et al.* 2003). I confirmed this using a two-way ANOVA, which demonstrated no significant difference in the NGDR (arcsine transformed to meet assumptions of normality) and speed of no-odorant controls and nontracking animals (NGDR: $F_{2,\,219} = 1.46,\ p = 0.23$; speed: $F_{2,\,219} = 0.0038,\ p = 0.99$) as functions of odorant level. For the no-odorant controls and nontracking animals substrate had significant effects on both speed and NGDR ($F_{3,\,219} = 9.83,\ p < 0.001$; $F_{3,\,219} = 3.52,\ p < 0.001$, respectively); animals tended to move faster and more directly upstream with rougher bed conditions, and there was a particularly dramatic effect at the roughest bed condition. Detailed observations indicate

Table 2.3. Summary of crab tracking behavior statistics, showing the effects being tested, the test statistic, associated degrees of freedom, and the resulting probability value for all analyses of the behavioral results.

	G	F	df	p
Hit x Bed Roughness	8.3	-	3	<0.05
Hit x Concentration	15.5	-	1	<0.05
Hit x Substrate x Concentration	11.28	-	6	0.082
NGDR x Control x Miss	-	1.46	2, 219	0.23
Speed x Control x Miss	-	0.0038	2, 219	0.99
NGDR x Substrate	-	9.83	3, 219	<0.001
Speed x Substrate	-	3.52	3, 219	<0.001
NGDR x Concentration	-	1.24	1, 83	0.2685
NGDR x Substrate	-	1.62	3, 83	0.19
NGDR x Substrate x Concentration	-	0.54	3, 83	0.66
Speed x Concentration	-	14.77	1, 83	<0.001
Speed x Substrate	-	3.87	3, 83	<0.05
Speed x Substrate x Concentration	-	0.59	3, 83	0.62
Distance from Centerline x Concentration	-	0.16	1, 240	0.689
Bed Roughness x Distance from Centerline	-	4.54	1, 240	0.004
Distance Downstream x Distance from Centerline	-	11.18	2, 240	<0.0001
Distance Downstream x Normalized Distance from Centerline	-	445.99	2, 240	<0.001
Normalized Distance from Centerline x Substrate	-	1.36	3, 240	<0.25

that crabs increase use of their swimmerets, particularly on the roughest substrate. This tends to reduce the degree of contact with the bottom, increasing speed and minimizing changes in direction.

Successful trackers behaved differently than unsuccessful trackers in their response to odorant and substrate treatments (Figure 2.2). A two-way ANOVA, examining the effects of concentration and substrate on NGDR of successful trackers (arcsine transformed to meet assumptions of normality) (Figure 2.1a), indicated that there is not a significant effect of either factor or their interaction on NGDR (concentration: $F_{1,}$

$_{83} = 1.24$, $p = 0.2685$; substrate: $F_{3, 83} = 1.62$, $p = 0.19$; interaction: $F_{3, 83} = 0.54$, $p =$ 0.66).

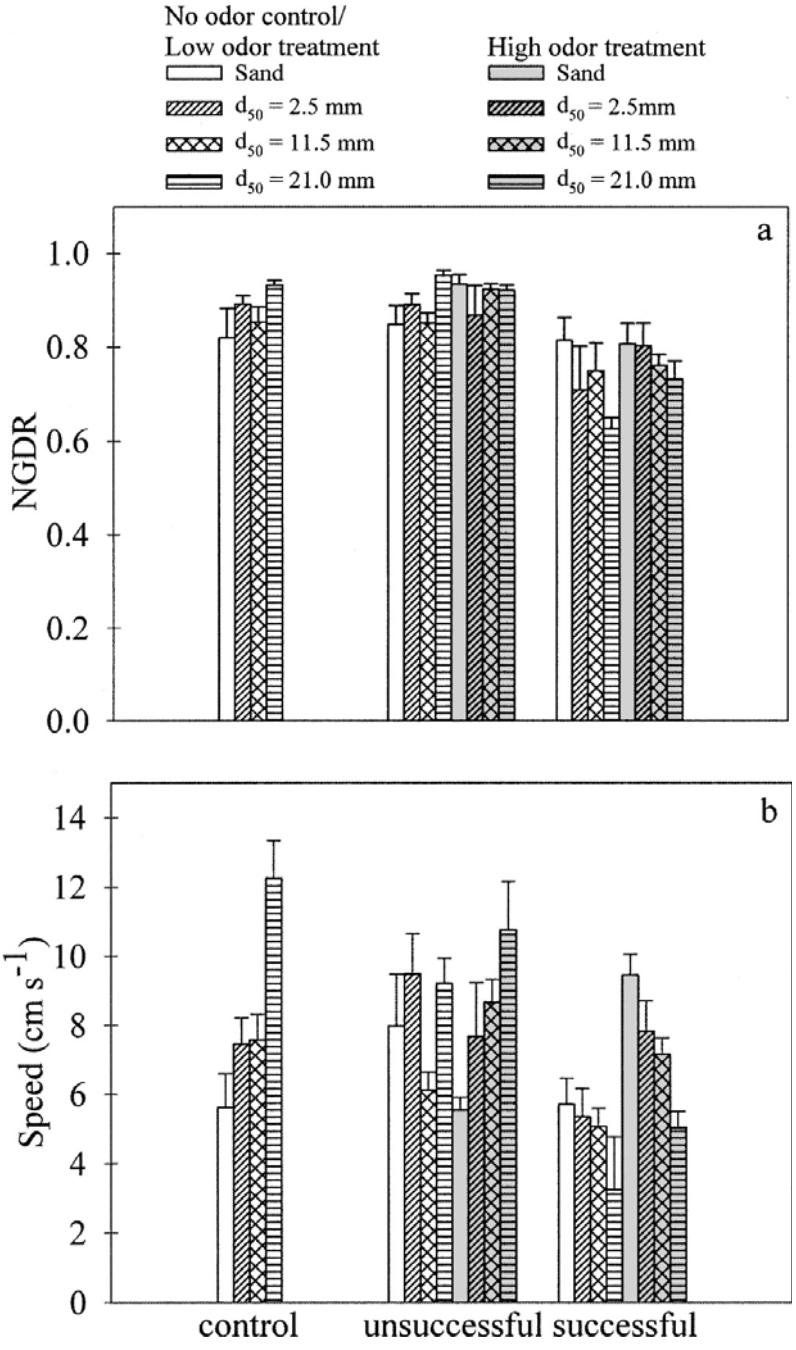

Figure 2.1. (a) Net to gross displacement ratio (NGDR) ± the standard error of the mean (SEM) for crabs in various odorant and substrate conditions. (b) Average speed ± SEM (cm s^{-1}) for crabs in various odorant and substrate conditions. The figure shows data for crabs in no-odorant controls, as well as for animals that missed or successfully hit the stimulus when foraging in four bed-roughness treatments (fine sand, d_{50} = 2.5 mm, d_{50} =

11.5 mm, and d_{50} = 21 mm) with two stimulus concentrations (low and high). Sample sizes are reported in Table 2, except that n = 31 for d_{50} = 11.5 mm at the high stimulus concentration since one path could not be fully recovered from the video tape.

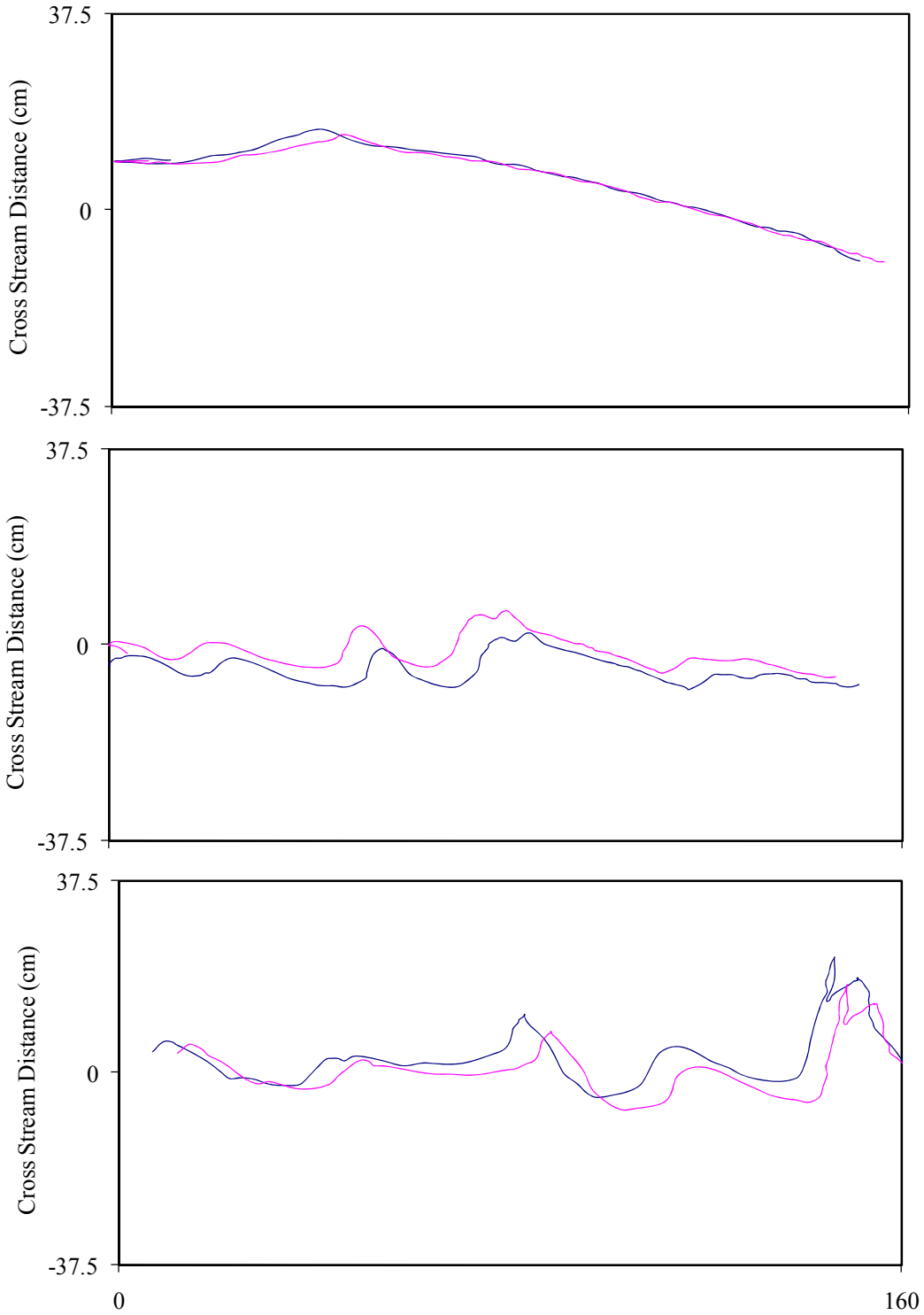

Figure 2.2 Typical tracks of animals that (a) unsuccessfully and (b, c) successfully located the odorant source (0, 0). In all cases, flow velocity is from left to right at 5 cm s^{-}

[1] and lines represent path plots for each of the LED's of the crab backpack. (a) Unsuccessful over substrate roughness d_{50} = 11.5; (b) successful over d_{50} = 2.5; (c) successful over d_{50} = 11.5.

In contrast, the speed of a successfully tracking crab was significantly reduced by both a decrease in concentration and an increase in substrate roughness (Figure 2.1b; concentration: $F_{1, 83} = 14.77$, $p < 0.001$; substrate: $F_{3, 83} = 3.87$, $p < 0.05$), with no evidence of a substrate-concentration interaction ($F_{3, 83} = 0.59$, $p = 0.62$).

Successful trackers appeared to maintain positions farther from the plume midline as substrate roughness increased, and they showed a general trend of increasing their transverse position at greater distances from the source (Figure 2.3a, where *d* is defined as the distance from the centerline). I determined whether odorant properties and bed roughness influenced the transverse location of the animal by examining the average distance from the plume centerline as functions of concentration, substrate, and distance from the odorant source (Figure 2.3a). This three-way ANOVA revealed that although there was no significant effect of concentration on distance from centerline ($F_{1, 240} = 0.16$, $p = 0.689$), there was a significant effect of bed roughness and distance downstream from the odorant source ($F_{3, 240} = 4.54$, $p = 0.004$; $F_{2, 240} = 11.18$, $p < 0.0001$, respectively). There were no other significant interactive effects.

As presented in Rahman and Webster (2005), transverse profiles of the time-averaged concentration field follow a Gaussian distribution shape, and, hence, the half-width of the profile is quantified by the standard deviation (σ) of the profile shape. I normalized the average distance from the centerline by the time-averaged plume half-width for each downstream region, and reanalyzed this data in order to detect self-similarity of tracking behavior (Figure 2.3b). The normalized distance from the centerline

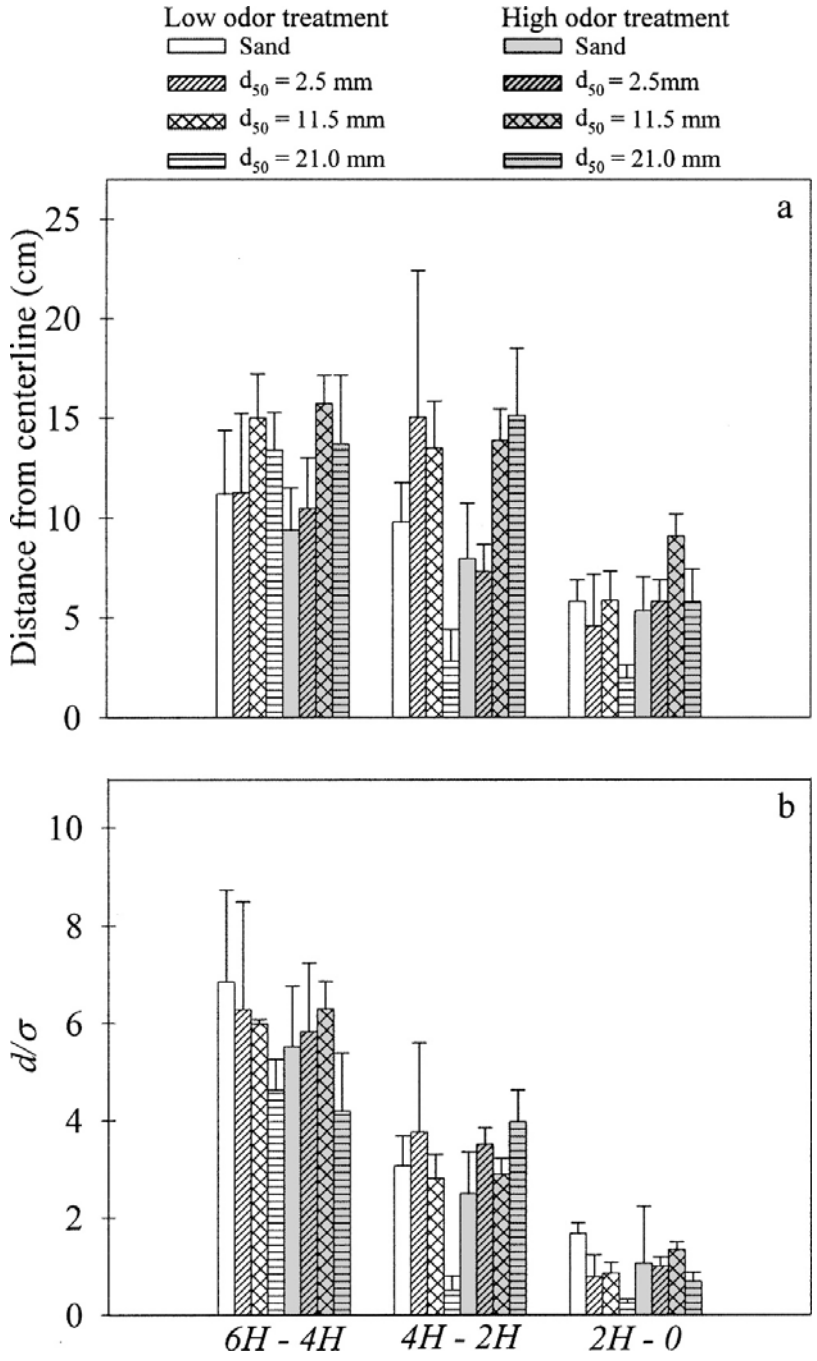

Figure 2.3. (a) Distance from centerline, d ± SEM (cm), as a function of distance from the source for crabs in various odorant and substrate conditions. (b) Distance from centerline, d, normalized by σ, the standard deviation of the transverse profile of the time-averaged concentration. The figure shows data for crabs that successfully hit the stimulus when foraging in four bed-roughness treatments (fine sand, d_{50} = 2.5 mm, d_{50} = 11.5 mm, and d_{50} = 21 mm) with two stimulus concentrations (low and high). H is the channel water depth and equals 0.2 m.

decreased significantly as animals approached the source, as before ($F_{2, 240} = 445.99$, $p < 0.001$). In contrast to the previous results, normalized distance is not significantly affected by substrate ($F_{3, 240} = 1.36$, $p < 0.25$), and there are no other significant main effects or interactions ($F < 0.69$, $p > 0.56$ for all effects). The mean normalized distance (*i.e.*, the location of the center of the crab) from the centerline across all substrate and concentration treatments was approximately 5.9, 2.7, 1.1 in regions $6 > x/H > 4$, $4 > x/H > 2$, and $2 > x/H > 0$, respectively.

2.4 Discussion

Prior research in both aquatic and terrestrial environments has demonstrated that hydrodynamics alter chemical signal structure and the performance of searchers that navigate through these odorant plumes (Weissburg 2000; Koehl 2006; Vickers 2006). The behavioral results presented in this study in conjunction with the plume measurements from Rahman (2002) and Jackson *et al.* (2007) corroborate other investigations of olfactory search in blue crabs and terrestrial arthropods by documenting decreased search success in increasingly turbulent conditions. Substrate roughness reduces the ability of blue crabs to find odorant sources by increasing boundary layer turbulence, which decreases the time-averaged and burst odorant concentration, homogenizes the plume, and increases the plume width. Previous studies on blue crabs have documented a sharp decrease in foraging success as the boundary layer assumes a transitional rough character (*e.g.*, Weissburg and Zimmer-Faust 1993). Data from the current study suggest another marked decline in foraging success as the boundary layer becomes fully rough.

Rahman (2002) continues in the tradition of previous studies that have examined aspects of the vertical structure (*e.g.*, Moore and Atema 1991) or used PLIF to examine isolated planes (*e.g.*, Webster and Weissburg 2001; Mead *et al.* 2003) using point sampling methods or planar fields that are limited in the vertical domain. While these studies are useful, Rahman's (2002) study along with Rahman and Webster (2005) improves our understanding of the information content in chemical plumes by providing a more comprehensive evaluation of the vertical and horizontal variation in signal structure across a range of boundary-layer turbulence conditions.

Although our hypothesis concentrates on the role of water column signal structure, bed roughness may play a role in advective and diffusive permeability of the stimulus into the substrate and, perhaps, modulate crab behavior. Prior research has demonstrated that diffusive transport dominates solute transport at the sediment surface (Glud *et al.* 1996), but significant advective pore-water flow can be induced by imbalances of pressure due to surface roughness elements (Thibodeaux and Boyle 1987). This suggests that advective flow will introduce increasing levels of the stimulus into the benthos with increased bed roughness, potentially to levels where tracking blue crabs could utilize these signals to help them find an odorant source. Control trials were interspersed with stimulus trials for all bed roughnesses; hence, I might have expected similar behavior for crabs under both conditions if stimulus were being built up and retained in the sediment at levels that modulated crab tracking. The difference between tracking and nontracking animals (and the similarity between animals that did not track and those moving in the absence of stimulus) suggests that, although crabs may detect pore-water signals during tracking, these potential signals alone do not appear to be

enough for a foraging crab to successfully locate a food source. Prior experiments (Weissburg and Zimmer-Faust 1993, 1994) have indicated that crabs are rarely successful in finding sources when chemical signals are introduced over natural substrates without ambient flow, further suggesting that pore-water signals have, at best, a minor role in mediating tracking.

The quantification of odorant plume properties suggests that the signal structure depends on the flow microenvironment in general and the vertical position in particular, and, consequently spatial variation in the odorant signal structure may be important in explaining how animals extract information from chemical plumes. Microenvironments, as used here, refer to regions within the general environment that correspond to an area of interest on an organism (Atema 1985). In this case, flow microenvironments were examined at the heights of the different sensors on the body of a blue crab.

As discussed in detail below, odorant signal properties in different regions of the boundary layer are well suited to control specific behaviors mediated by sensory appendages that sample odorants within those regions. Prior research has revealed that organism's sensory systems may be specifically adapted, or optimally tuned, for their general environmental conditions (Bodznick *et al.* 1999; Bleckmann *et al.* 2003; Warrant *et al.* 2003); however, this study suggests that parts of an organism's sensory system may be tuned to their particular microenvironments and behavioral roles. Because sensory neuron properties often match the spatiotemporal scales of variation of the relevant natural stimuli (Rieke *et al.* 1997; Machens *et al.* 2001), it logically follows that individual chemosensors/appendages of organisms exposed to different signal properties may be tuned for their particular flow microenvironments.

2.4.1 Effects of vertical variation of the concentration field on signal structure at the antennules

The data indicate a significant negative relationship between bed roughness and speed of tracking animals (Figure 2.1b). Tracking speed is largely determined by upstream motion, which is mediated by sensory neurons in the antennules and antennae (Keller *et al.* 2003). These centrally located organs sample higher in the water column than the leg chemosensors and therefore are presumably subjected to different plume signal properties. For instance, Rahman (2002) found significant influence of bed roughness on the vertical structure of time-averaged plume concentration (*see* Figure 4, Jackson *et al.* 2007), thereby significantly affecting the concentrations available for sampling at the height of the centrally-located antennules.

Although the influence of bed roughness on the time-averaged concentration is significant, presumptive chemosensory cues relate more to the spatial distribution of odorant and the concentration fluctuations. Vertical profiles of the standard deviation reveal a decrease in concentration fluctuations with increased roughness and an overall trend of greater plume homogeneity close to the bed as compared to higher in the water column (Figure 5, Jackson *et al.* 2007). This indicates that intense odorant bursts are less likely to occur with greater levels of substrate-induced mixing.

Intermittency is another plume characteristic that shows significant variation at the height of the antennules. Rahman (2002) calculated an intermittency factor (γ), defined as the probability of the instantaneous concentration exceeding a prescribed threshold (Chatwin and Sullivan 1989):

$$\gamma = \text{probability}\,[c(x,t) > C_{th}]$$

The metric describes the probability that a filament with concentration (c) greater than the threshold (C_{th}) will be present at the measurement location. The choice of threshold is arbitrary, and the results, which are highly threshold dependent, must be interpreted with respect to the threshold choice. Jackson *et al.* (2007) presented vertical profiles for two threshold definitions: 2% of the source concentration, 0.02 C_0, and the local time-averaged concentration, $\overline{c}$ (*see* Figures 6 and 7, respectively). In the first case, the threshold is constant throughout the field and hence provides insight to the probability of filaments of high absolute concentration. In the second case, the threshold varies with position and provides insight to the probability of filaments of high concentration relative to local conditions. The results for the fixed threshold are easier to interpret, whereas the variable-threshold results are perhaps more relevant for biological sensors, which often detect intensity changes as opposed to absolute intensity (*e.g.*, Gomez *et al.* 1994).

Vertical profiles of the intermittency factor based on a threshold of 2% of C_0 indicate that the probability of encountering concentrated odorant filaments is greatest close to the source, at intermediate water depths, and in conditions of low turbulence created by smoother substrates. The intermittency factor decreases with increasing bed roughness and increasing distance from the source due to increased dilution of the chemical filaments to concentration levels below the absolute threshold.

The intermittency factor, with a threshold defined by the local time-averaged concentration, would equal 0.5 if the distribution were symmetric; that is, half of the samples exceed the average in a symmetric distribution. Hence, the intermittency factor based on a threshold of the time-averaged value provides a simple measure of the distribution skewness as well as the probability of a concentration sample that exceeds

the local mean. Webster and Weissburg (2001) demonstrated that the distribution of the instantaneous concentration value is not symmetric in the plume; hence, I anticipated that the intermittency would differ from 0.5. At the x/H = 1.0 location, profiles for all bed-roughness treatments are fairly uniform in the vertical direction with a value of roughly 0.2; thus, 20% of the samples exceed the local time-averaged value (*see* Figure 4 from Jackson *et al.* 2007), independent of the vertical position and the bed roughness. As the plume evolves downstream, the intermittency factor for the two rougher bed conditions is greater than the intermittency factor for the smoother bed conditions; the probability that a sample exceeds the local time-averaged value is greater for the rougher beds. The profiles also become less vertically uniform with larger intermittency closer to the bed. To summarize, the results of examining both standard deviation and intermittence indicate that plumes evolving over smoother substrates are characterized by more intense, but less numerous odor filaments.

Together, these results indicate a decreased probability of encountering concentrated odorant signals at the height of the antennules as turbulence increases. The corresponding decrease in the speed of tracking blue crabs likely was caused by the decreased frequency of intense odorant bursts since the mean concentration is not easily resolved (Webster and Weissburg 2001).

The potential role of neuronal adaptation also must be evaluated as a possible component of the sensory mechanism. Sensory adaptation renders neurons unresponsive to background stimuli (Atema 1985), suggesting that signal contrast, as opposed to absolute signal level, is important. The intermittency factor, expressed as the probability of exceeding a fixed threshold based on the initial concentration, decreases at the height

of the antennules as increased bed roughness creates a more homogenous plume (*see* Figure 6, Jackson *et al.* 2007). However, the probability of bursts with concentration above the local time-averaged value increases with increasing bed roughness. At the height of the antennules, the probability of a burst above the time-averaged concentration is greater for increased bed roughness, but the standard deviation is much less (*see* Figures 7 and 5, respectively, Jackson *et al.* 2007). Hence, fluctuations greater than the time-averaged value are more common, but not particularly large in concentration. Again, the observation that walking speed decreases with increased bed roughness is consistent with the hypothesis that upstream motion is mediated by large instantaneous concentration (bursts) at the antennules. The data further suggest that it is the intensity of the bursts, rather than the frequency of bursts above background, that bears the greatest relationship to upstream movement.

2.4.2 Effects of transverse variation of the concentration field on the signal structure at the appendages

Chemosensors on the walking legs are responsible for the turning motion that keeps blue crabs within a chemical plume (Keller *et al.* 2003). Spatial separation of leg chemosensors across the body allows perception of instantaneous stimuli contrasts transversely across the body if the sensor spacing is large enough. The ability of spatially separated sensors located close to the substrate to encode information on plume spatial structure may be enhanced by vertical variation in the odorant field structure, at least under some circumstances. Close to the source ($x/H < 2$) and in conditions of smoother bed roughness, the plume structure close to the bottom is more homogeneous (*i.e.*, lower standard deviation observed in Figure 5 from Jackson *et al.* 2007) compared to the plume

structure at the height of the antennules. The probability of encountering filaments with concentration exceeding the local time-averaged concentration is greater close to the bed as long as $x/H > 1$ (*see* Figure 7 from Jackson *et al.* 2007), which also facilitates encoding of spatial information. Perhaps unsurprisingly, there was very little evidence that turbulence affected the ability of foraging crabs to steer themselves with respect to the plume (Figure 2.1). This may be specific to the geometry of the odorant release, as other investigators have observed that variation in turbulent mixing conditions induced substantial changes in steering (Weissburg and Zimmer-Faust 1993, 1994) when the odorant source consisted of a vertical jet. Nonetheless, our current results suggest that upstream progress and steering may not be equally affected by varying turbulence and that these two processes need to be considered separately when discussing impacts of the fluid environment on chemosensory navigation.

Some aspects of spatial orientation are affected by the bed roughness manipulations. Because rough substrates cause greater spreading of the plume as it evolves downstream (*see* Figures 2, 3, and 8, Jackson *et al.* 2007), there are corresponding significant effects of bed roughness and distance from the source on the average distance that the crabs wandered from the centerline (Figure 2.2a). These correlations add weight to the suggestion that blue crabs mediate their turning by perceiving transverse contrast (*i.e.*, contrast across the plume width) in the chemical signal by using bilateral comparison across the body (Zimmer-Faust *et al.* 1995, Keller *et al.* 2003).

Jackson *et al.* (2007) used the transverse (across the width of the plume) integral length scale, L, to provide a measure of the plume width and homogeneity. The integral

length scale is calculated by integrating the area under the normalized transverse correlation function of instantaneous concentration samples. Bed roughness increases the integral length scale because the plume width and homogeneity increase with rougher substrates (*see* Figure 8, Jackson *et al.* 2007). The normalized curves of the transverse spatial correlation of concentration are presented in Jackson *et al.* (2007) for sensor spacing lengths of *L* and 10 cm (roughly equivalent to the span between tips of the walking legs of our animals). With a sensor spacing of *L* (*see* Figure 9a, Jackson *et al.* 2007), the curves collapse onto the same trend irrespective of the bed roughness or downstream location. This indicates that the scales chosen for nondimensionalization, the transverse integral length scale for the correlation spacing and the time-averaged plume width for the position of the inner sensor, correctly characterize the transverse correlation (and contrast) of the intermittent plume structure. With a sensor spacing of 10 cm (*see* Figure 9b, Jackson *et al.* 2007), there is a monotonic trend with increasing *x*. The value of the correlation function depends on the bed roughness treatment, which eliminates the self-similar pattern from a sensor spacing of *L*, and the correlation function for the distance of the interior sensor to the centerline (normalized by σ) reaches a very low value for y/σ greater than approximately 1.5 for all roughness cases.

Greater transverse spread of the plume requires that searchers either increase their sensor span (*i.e.*, increase their sensor span in proportion to *L*, Figure 2.3a) or move farther from the plume centerline in order to experience asymmetric odorant stimulation (Figure 2.3b). Webster *et al.* (2001) suggested that a searcher with sensors separated by a distance larger than *L* can better assess contrast between sensors and therefore identify the direction towards the plume centerline more easily. My results indicate this is a

general finding that remains true even with increasing bed roughness: sensor spans greater than *L* showed diminished correlation of signal intensity received by each sensor. Nevertheless, increased distance from the plume centerline is the most likely effect of increased plume width since the ability to enlarge sensor span is limited to the maximal appendage spread. The decrease in average centerline distance as foragers move towards the source is a natural consequence of the reduction in plume width relative to the sensor span.

Changes in the average distance to the centerline across substrate roughness experiments suggest how the path to the source has similar directness across the bed treatments despite changes in plume width (Figure 2.1a). Blue crabs appeared to be reacting to the change in plume width and homogeneity by moving farther from the centerline of the plume (Figure 2.2a) to enable efficient tracking. The benefit is illustrated in Figure 2.3b, which shows that the contrast between sensors with a fixed transverse separation decreases as the sensors move farther to the side. Hence, blue crabs appear to maintain their steering ability by moving more to the side of the plume where they can perceive a greater contrast.

One challenge for understanding perceptual mechanisms is to determine if differing behavior in various environments reflects a change in sensory strategies or the application of a constant rule that results in behavioral differences across environments. In an effort to disentangle these two possibilities, I normalized the centerline distance to the half-width of the time-averaged plume, σ (Figure 2.2b). In analogy to self-similarity of physical processes (*e.g.*, Figure 2.3a), I sought to determine if there was a fixed

relationship of sensor positions to a measure of plume width and whether this relationship revealed possible constancy of underlying rules employed by foraging crabs.

This analysis revealed both variant and invariant components. Scaling the average crab position relative to the centerline by σ removed dependence of the distance of the crab to the centerline on bed roughness. This suggests that sensor positions of foraging animals (at a given position downstream) bear a constant relationship with plume width. Conversely, the average distance to the source remained unchanged by non-dimensionalizing the results by σ. That is, blue crab trackers seemed to employ rules that fixed the contrast between sensor pairs as a function solely of downstream distance of the source; animals in similar plume regions maintained a fixed distance from the plume centerline relative to σ regardless of substrate roughness. This normalized distance declined as animals moved toward the source, indicating an increase in the contrast between sensors may be part of the mechanism that maintains tracking.

One of the most powerful implications of self-scaling of behavior and plume properties is that these relationships allow us to compute the average correlation experienced by sensor pairs as the animal moves towards the source. As presented in Jackson *et al.* (2007), scaling the sensor span to the integral length scale results in self-similar correlation function profiles. This scaling of the plume structure suggests that variable sensor spacing is advantageous since L changes with distance to the source. In contrast to this hypothetical advantage, the chemosensors on the leg appendages of blue crabs are relatively fixed. Comparing the results of the 10 cm fixed spacing of the normalized transverse spatial correlation curves of concentration to the behavior results suggests that animal position results in consistently low correlations among spatially-

separated sensors on the walking legs (Figure 2.3b), which are the presumptive arbiters of steering (Keller *et al.* 2003). Far from the source ($x/H > 5.3$), it is possible to maintain a position near the center of the plume where sensors receive correlated input. Despite this, and the fact that animals initially started close to the plume centerline as a result of being held within a cage, they assumed an average position during tracking that resulted in low correlation and high contrast. Although the average distance of the animal from the centerline decreases, interior sensors are always located such that the correlation between sensors with a 10 cm spacing is not particularly large, which implies that there continues to be high contrast between sensor pairs across the body axis of tracking crabs.

The combined analysis of signal correlation and behavior supports an edge-steering mechanism possibly acting in combination with other strategies. Both L and σ are small close to the source (*see* Rahman and Webster [2005] for σ) because the plume is initially narrow, so a large fixed sensor span never results in appreciable correlation when animals are within 50 cm ($x/H < 2.5$). The observation that crabs move closer to the centerline in this region suggests the existence of at least one other rule governing steering behavior in addition to maximizing signal contrast. One possibility relates to the fact that the transverse correlation is uniformly low close to the source; therefore, any position in the plume is sufficient for steering when combined with information obtained from the interior sensor. In Figure 2.3b, the correlation function at $x/H = 2.7$ is small for all sensor locations, which results from the fact that the 10-cm sensor spacing is large compared to L. Hence, the need to remain near the "edge" of the plume to perceive a contrast is reduced, and the presence of odorant signal in this narrow plume region may be sufficient to indicate that the plume source is directly upstream and minimize the importance of

bilateral steering. Another possibility is that the crabs compare among different combinations of sensors as the plume width decreases. Figure 2.3a demonstrates that adjustable sensor spacing is advantageous in the self-similar plume structure. If the sensor span exceeds L, the contrast is high at all locations in the plume. Close to the source, the separation distance between the antennules and leg chemosensors becomes large compared to L, and contrast between these appendages could potentially be used for comparative steering. Regardless of the tracking strategy, it is clear that the signal properties used by foraging blue crabs are strongly related to the extent of the transverse variation, since the distance to the centerline collapses across turbulence treatments when normalized to σ.

2.4.3 The importance of 3D structure

Analysis of planar odorant fields at several heights above the bed illustrates the complexity of the three-dimensional odorant plume structure. In particular, this study demonstrates that the plume-tracking behavior of blue crabs is related to the chemical plume information available to their chemosensors at differential heights in the water column. A full appreciation for both behavioral/sensory strategies and the role of mixing processes is thus contingent on a more complete characterization of the odorant plume structure than what two-dimensional analysis can provide. Quantifications of the three-dimensional instantaneous concentration field around tracking animals may be enlightening because they capture the correlation and contrast between signals at various vertical regions. Rahman's (2002) analysis cannot resolve the temporal relationships of structure at various elevations because a series of odorant fields were collected through time at a specific height, measurements were then repeated across a series of elevations to

ultimately yield a time-averaged reconstruction of the plume structure. I suspect that changes in the vertical structure are an important element of navigational strategies and may possibly mediate the switch to near-source searching behavior. Simulations of odorant tracking utilizing planar concentration data (Weissburg and Dusenbery 2002) can consistently replicate many features of movement to the source but often cannot account for the ability of animals to identify the source location without overshooting it. Understanding the true 3-D nature of odorant signals appears to be necessary for a full description of chemosensory-mediated search behavior in boundary layers.

CHAPTER 3

MATERIALS AND METHODS FOR SIMULTANEOUS 3DLIF SYSTEM

The remaining chapters of this thesis involve the data collected from three-dimensional plume concentration measurements surrounding actively tracking blue crabs. The complex nature of this research necessitated collaboration with engineers to develop a system that could simultaneously record both plume characteristics and tracking behavior. The methods described in this section were developed collaboratively with Brian Dickman (Dickman 2008), a graduate student in Civil and Environmental Engineering. Brian was the principal designer of the three-dimensional LIF system, including the scanning laser, camera, related electronic circuitry, calibration, and image processing, while I primarily focused on behavior trial protocols and the subsequent analysis of crab tracking behavior. Although I will give a brief review of the method for quantifying odor, the details of this method are presented in Dickman *et al.* (2009). The major emphasis of this chapter is the behavioral methods and the subsequent analysis of crab tracking behavior in light of the plume measurements.

3.1 Introduction

The tracking behavior of organisms navigating in turbulent plumes traditionally has been studied by placing an animal in a controlled environment, releasing an attractant odorant upstream or upwind, and observing the resultant tracking behavior. This tracking behavior subsequently is related to plume properties collected under similar experimental

conditions to make inferences about the mechanisms behind the behavioral decisions. These types of experiments have revealed a great deal about generalized behavioral responses to turbulent odorant plumes but they fall short of providing a true quantitative measurement of the olfactory stimuli that govern tracking behavior, because incoming stimuli and the resulting behavior are not observed simultaneously.

As previously stated, the instantaneous 3D structure of odorant plumes is complex and there is great variation in plume characteristics accessible to tracking animals in all three dimensions (*i.e.*, cross-stream, down-stream, and with depth [Rahman and Webster 2005]). In particular, previous studies (Chapter 2, Jackson *et al.* 2007) have demonstrated that the plume-tracking behavior of blue crabs is related to the chemical plume information available to their chemosensors at different heights in the water column. It follows that measurement of the true 3D nature of odorant signals experienced by an organism is necessary for a full description of chemosensory-mediated search behavior in boundary layers. To accomplish this task, odorant concentration reaching the olfactory organs must be measured at spatial and temporal scales that are consistent with organism sensing abilities. The measurement technique must also be minimally intrusive because physical probes disturb the flow and often collect data only at a single point, which is not representative of organism's spatial sensing ability. Finally, the odorant cue structure needs to be collected simultaneously with animal tracking. To provide a comprehensive and useful characterization of odorant plume structure as an animal tracks to a source, I utilized a three-dimensional laser induced fluorescence (3DLIF) system in a seawater laboratory flume to generate 3D, time-

resolved characteristics of odorant plume structure with simultaneous behavior measurements of actively tracking blue crabs, *Callinectes sapidus*.

3.2 Experimental set-up

3.2.1 Flume system

Simultaneous odor signal quantification-tracking experiments were all conducted in a recirculating saltwater flume housed in the Civil Engineering Hydraulics Lab at Georgia Tech. The clear, acrylic tank of the flume measured 0.76 m wide by 13.5 m long with a roughly 2 m working section at the downstream end where the boundary layer was fully developed. Water was maintained at a depth of 21.2 cm by a weir and recirculated at a mean flow speed of 5 cm s^{-1}. The bed of the flume was covered in sand with an approximate average diameter (d_{50}) of 1 mm. These conditions were chosen to approximate conditions that blue crabs would realistically encounter in the field while foraging (Finelli *et al.* 1999; Smee and Weissburg 2006) and resulted in a boundary layer with a roughness Reynolds number (*Re*) of ~ 3 and a bed shear velocity (u^*) of 3.01 mm s^{-1} (Jackson *et al.* 2008).

3.2.2 Odorant

The attractive odorant stimulus was created by soaking 2.21 g of shrimp in 1 L of seawater for 1 hour, with fresh solutions made for each day of testing and every 2-3 hours within that day. This general preparation has been used in many behavioral assays involving aquatic organisms and, in particular, blue crabs have effectively tracked sources of chemical exudates of shrimp in previous behavior trails (Keller *et al.* 2003; Jackson *et al.* 2007). The specific concentration of stimulus was chosen as a value in the

middle of the dose response range of blue crabs. This created an odorant plume that crabs could easily follow under continuous, isokinetic release conditions, yet allowed us to see marked changes in behavior with plume dilution due to introduced turbulence.

The stimulus solution was mixed with a fluorescent dye, Rhodamine 6G, which served as visual proxy for the relative concentration of the attractive odorant. The Schmidt number (the ratio of viscosity to mass diffusivity of a solution) of Rhodamine 6G is approximately 1250 and is of the same magnitude of the Schmidt number of the odorant solution, indicating that the dye is a good representation of the location and concentration of the attractive odorant filaments. The combined solution was neutrally buoyant, non-reactive, and transported passively by the flow.

A stimulus delivery system was established in the working section of the flume that utilized a 4.2 mm diameter nozzle suspended 25 mm above the substrate (Figure 3.1). Placement of the nozzle just off the substrate in the inertial region of the turbulent boundary layer is ecologically relevant as it mimics a reasonable height of carrion sitting on the benthos that would be leaking attractive chemicals. The height is also representative of the approximate siphon height of hard clams (*Mercenaria mercenaria*), one of the main prey items of blue crabs. While actively pumping clams release odorants as a jet, clams often extend their siphons without pumping and it is currently unclear whether they passively give off cues in this state. The piping for the nozzle higher in the water column was baffled using a streamlined faring that minimized wake disturbance from the tube and therefore the potential disturbance of the stimulus itself. The location of the nozzle tip was centered cross-stream in the flow and was considered the x-y origin point (0, 0) of a coordinate system with the x-axis in the along-stream direction (positive

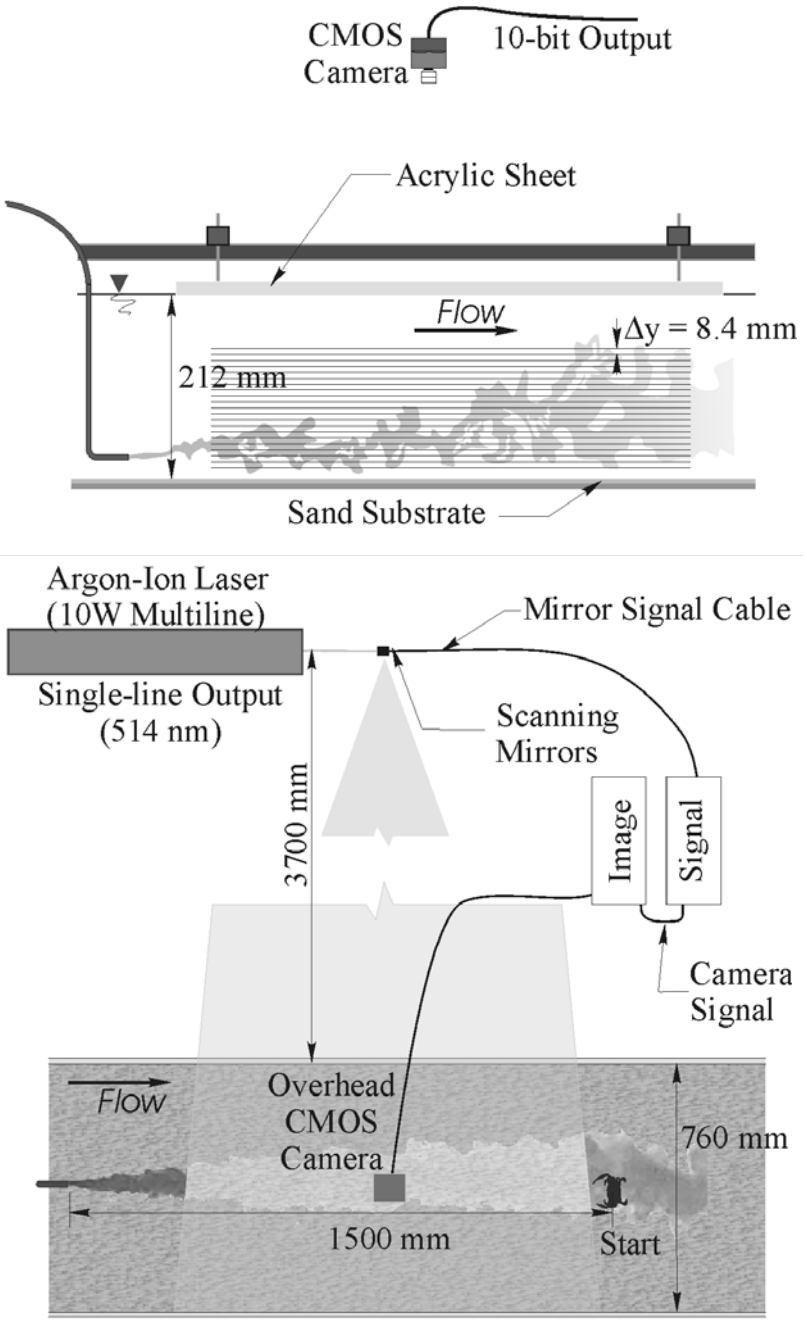

Figure 3.1. Configuration of the 3DLIF system, as seen from (a) the side of the flume and (b) above the flume. The horizontal lines in part (a) represent the sequential locations of the laser scan. *Figure borrowed with permission from Dickman (2008).*

x downstream) and the y-axis in the cross-stream direction (positive y towards laser). The substrate surface was considered the origin of the z-axis as depth (positive z towards water surface).

Three plume types were created: Continuous, Meandering, and Pulsed release. The three source release types enable spatial and temporal comparisons of plume structure with resulting behavior and are representative of realistic plume types available to blue crabs in the environment. Specifically, the Continuous plume provided basic comparisons of plume properties with resulting behavior, which could then be compared to the other plumes. This plume type is representative of a piece of detritus or carrion leaking chemicals on a mud or sand surface in an estuary. The Meandering plume was used to test the effects of large-scale spatial intermittency and the Pulsed plume was used to test the effects of large-scale temporal intermittency on crab tracking behavior. A continuous plume type could be made to meander by any upstream obstruction of flow, such as vegetation (*e.g., Spartina alterniflora* blades) or rocks, and a pulsed jet plume is similar to that released by an actively pumping clam (*e.g., Mercenaria mercenaria*). The release conditions for both the Continuous and Meandering plumes were created by the uninterrupted, isokinetic release of stimulus from the nozzle. Meander in the second plume type was induced by a 10.1 cm cylinder placed 50.8 cm upstream of the nozzle, thereby creating a von Karman vortex street that generated turbulence in the cylinder wake. The Pulsed plume was released at a frequency of 0.1 Hz created by a 5 second odorant pulse followed by a 5 second period of no odorant. The odorant pulse was released with a slight momentum relative to the surrounding flow in order to match the

scalar mass release rate of the other two plumes (*see* Dickman [2008] for fluid dynamical characterization of the three plume environments).

3.2.3 3DLIF system

Laser-induced fluorescence (LIF) permits non-intrusive quantitative measurement of instantaneous concentration fields (Crimaldi 2008) by utilizing low concentrations of a tracer dye (*i.e.*, a light-reactive fluorophore) to illuminate plume structure. The fluorophore excites on a molecular level when hit by laser light and, when added to an odorant plume, serves as a proxy for odorant concentration provided the odorant and tracer diffuse at the same rate and decay minimally. This technique is minimally invasive and does not disturb the flow as introduced probes would. A 3DLIF system was set up alongside the working section of the flume (Figure 3.1) to measure the concentration of the odorant filaments. The system consisted of an Argon-ion laser, a pair of orthogonally mounted mirrors, and an overhead camera, all controlled by a computer system running Video Savant software.

Rhodamine 6G was specifically chosen as the fluorescent dye for these experiments as its peak absorption wavelength is 530 nm, which is close to the wavelength of the laser illumination (514 nm), and its resistance to photo-bleaching (Crimaldi 1997; Larsen and Crimaldi 2006). The transparent sidewalls of the flume allowed optical access to the test section and a similarly transparent, polyacrylic sheet was suspended at the water surface to eliminate refraction effects from small surface waves during calibrations and experiments.

The orthogonally mounted mirrors were used to scan the laser beam across the test section in both the horizontal and vertical directions. One mirror progressively

advanced the laser beam horizontally, thereby essentially creating an "instantaneous" two-dimensional "sheet" of light. The other mirror shifted the laser beam vertically at the end of each horizontal scan prior to the initiation of the next horizontal scan. This sequentially illuminated a three-dimensional volume created by 20 horizontal scans, each separated vertically by 8.4 mm. The lowest and highest scan positions were located at 0.5 cm and 16.8 cm above the substrate at the flume centerline, respectively, and were chosen to encompass the extent of the chemical plume within the sampling volume with the lower limit being set by the approximate level of *C. sapidus* leg chemosensors. The horizontal limits of the laser scans stopped 40 cm downstream of the source and therefore no data was collected in this region. References to Total path data refer to data from 150-40 cm downstream of the source while data analyzed by Section of the plume refer to regions 150-100 cm (downstream), 100-50 cm (middle), and 50-40 cm (upstream) downstream of the plume source.

The overhead camera (Mikrotron model MC1302) was outfitted with an optical filter (Tiffen Orange 21 with a cutoff wavelength of approximately 560 nm) to pass fluoresced light to the camera sensors while eliminating scattered laser light. A standard dielectric (hot) mirror was used to protect the sensors from infrared light. The camera lens allowed 1 mm pixel resolution at an elevation of 50 mm above the channel substrate. The resulting depth of field allowed an adequate level of focus for each of the 20 scan elevations that were collected by the camera at a rate of 100 frames per second. The camera acted as the timing master, sending a signal that initiated scanning of the laser by the mirrors. The resulting 3DLIF image data was collected with Video Savant software

at a collection rate slightly less than 5 Hz (*i.e.*, the 20 planes required 0.21 seconds to collect).

The 3DLIF system was calibrated with 10-12 concentration levels of Rhodamine 6G mixed in seawater (0 to 100 μg L^{-1}). The laser was scanned through the tank at experimental elevations and the resulting fluorescent signal was corrected for the effects of signal attenuation, magnification, refraction, and noise in the camera signal among other factors. The calibration resulted in a linear relationship between concentration and the camera pixel intensity for concentrations below 60-80 μg L^{-1} with an uncertainty of ± 3%. Additional details on the setup and calibration of this system are provided in Dickman *et al.* (2009).

3.2.4 Behavior measurements

Crab behavior was observed in all three plume types: Continuous release (number of successful tracks recorded, N = 15 tracks), Meandering release (N = 13 tracks), and Pulsed release (N = 12 tracks). Blue crabs were collected and maintained as outlined in Chapter 2, Section 2.3, and mean crab size did not statistically differ over the three treatments (Continuous = 13.85 ± 0.4231 cm; Meandering = 14.76 ± 0.3263 cm; Pulsed = 14.35 ± 0.2827 cm; $F_{2,36} = 1.6319$, $p = 0.2097$). From preliminary observations, it was clear that *C. sapidus* displayed an adverse reaction to the laser light causing them to either avoid walking through the laser sheet or move through it as quickly as possible. Thus, crabs were reversibly blindfolded using electrical shrink-wrap tubing to eliminate this visual disturbance. These blindfolds were secured to the eyestalks by application of heat and were generally removed by the crabs within a week of being blindfolded. Crabs were allowed to become accustomed to the blindfolds for a minimum of 48 hours before

being used in behavior trials. Initial experiments indicated that the shrink-wrap was sufficient to effectively block the laser light and behavior of blindfolded crabs was not significantly different than the behavior of non-blindfolded crabs when tracking shrimp stimuli of 2.21 $gL^{-1}hr^{-1}$ over sand in 5cm/s free channel flow. This conclusion is supported by examining kinematic behavior during tracking (t-test assuming equal variances), which showed that net-to-gross-displacement-ratio (path linearity) was not significantly different (0.856 ± 0.144 [mean ± stddev] and 0.814 ± 0.101 for un-treated blindfolded crabs, respectively; $p = 0.44$, $N = 13, 14$, respectively); and similarly, that walking speed did not differ between un-treated and blindfolded crabs (speed = 7.394 ± 2.010 cm s^{-1} and 5.713 ± 2.612 cm s^{-1} for un-treated and blindfolded crabs, respectively; $p = 0.14$, $N = 13, 14$, respectively). These values are comparable to those observed over many experiments with blue crabs in similar behavioral trials (Weissburg and Dusenbery 2002, Keller *et al.* 2003, Jackson *et al.* 2007). Percent success of crabs with blindfolds was again similar to crabs without blindfolds with un-treated crabs achieving 40% success (*see* Jackson *et al.* 2007) and blindfolded crabs achieving 46% success of finding the stimulus (N = 7 out of 13).

Behavioral trials were set up similarly to those conducted in the bed roughness trials (*see* Chapter 2, Section 2.1). Crabs were outfitted with a laser-emitting diode (LED) backpack to indicate their position, which was recorded by the CMOS camera simultaneously with the Rhodamine 6G fluorescence data. The flume area was darkened during trials to increase the contrast of the emitted light from the fluorescent dye and the backpack lights compared to the background light level. Crabs were acclimated in a cage at the downstream end of the test arena for 10 minutes. During this time, odorant plume

release was initiated to ensure that a developed plume would be present for the entirety of the experiment and that crabs would have contact with the stimulus plume prior to entering the test section. The laser and data collection system were initiated prior to the crab exiting the acclimation cage. Once the cage door was raised, crabs were given 10 minutes to initiate tracking by exiting the cage and an additional 10 minutes to complete their motion across the test section once they departed the cage. Crabs either moved through the test section and missed or found the odorant source within this time period, or remained stationary within the cage for the entire time period. No animals were in the process of tracking at the end of the observation period.

3.3 Data collection and analysis

3.3.1 Crab position

The bright spots on the recorded 3DLIF images created by the individual marker lights were easily distinguished from the fluorescence (Figure 3.2). LED coordinates were set as the center of these bright spots and these coordinates were subsequently examined for validity during post-processing by visually comparing the calculated trajectory of the marker lights with their actual trajectory in the raw image data. Because the LEDs did not require contact with a laser beam to emit light, they were present in all frames collected by the camera. Raw crab position data were therefore acquired at a higher rate than fluorescent data and were subsequently downsampled to the concentration data collection rate of roughly 5 Hz.

There was a substantial amount of reflected laser light from the backpack and the crab carapace and claws that was recorded by the CMOS camera along with the LED and

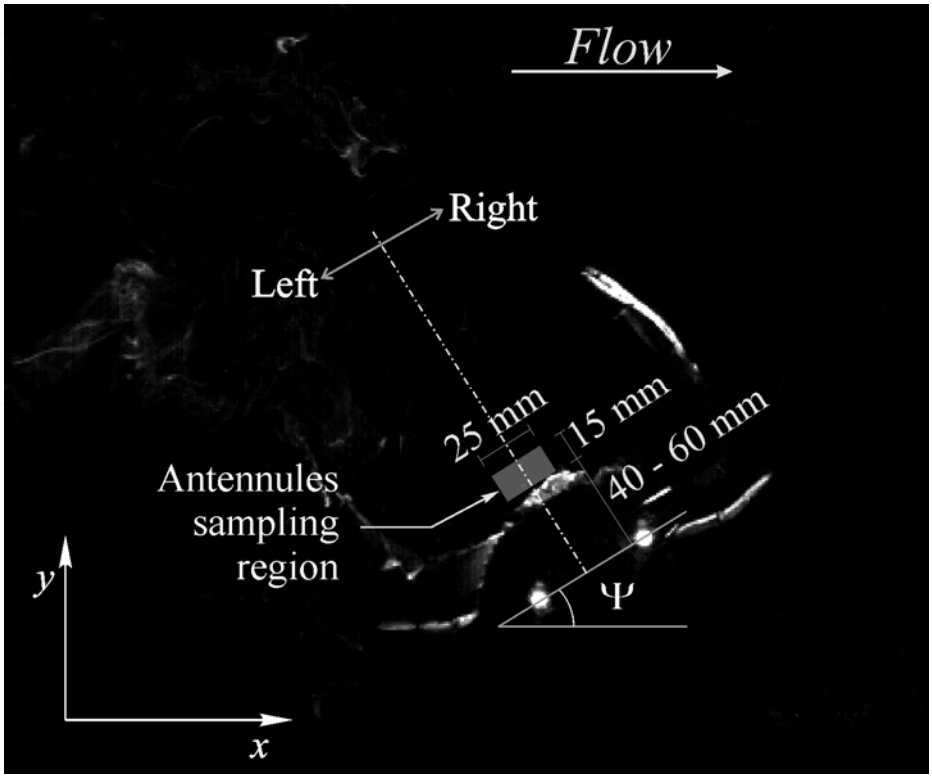

Figure 3.2. Location of the sampling zone for the crab antennules region. The two bright dots in the lower right region of the image are the light-emitting diodes (LEDs) used to quantify the crab position. Light reflecting from the carapace and mouth parts is observed in the space between the bright dots and antennules sampling region. ψ is the crab orientation angle with respect to the x-axis (and mean flow direction). The sampling box is a standard 25 mm by 15 mm oriented at the angle ψ. *Figure borrowed with permission from Dickman (2008).*

fluorescence light. These sources of light noise created by the crab's body also governed the subsequent analysis methods developed to collect odorant concentration data for successful searches. The crab body also created considerable shadowing of the plume as the laser was directed from a single side. Only crabs that tracked the plume leading with their left claw (*i.e.*, facing the laser) were able to be used for analysis due to these limitations.

Several variables were derived from the crab position information that were used in analysis (Figure 3.2). A line drawn between the two LED's (across the widest axis of the crab carapace) was compared to the x-axis to result in the crab's angle of orientation

with respect to the flow (ψ). The point equally bisecting the distance between the LED's along this line was considered to be the center of the crab (X_{crab}, Y_{crab}), which was used to determine the distance of the crab from the centerline of the plume (d). The crab's velocity was recorded in both the x and y directions (V_x and V_y respectively) and the combined change in position with time was noted as the Total velocity (V_T); acceleration was derived from those velocity values (A_x, A_y, A_T, respectively). Positive velocity or acceleration values in the x direction indicate motion towards the source (upstream), while negative values indicate motion away from the source (downstream). Positive y-values indicate cross-stream motion to the right of the plume midline and negative y-values represent cross-stream movement to the left of the plume midline. Turns were defined according to three criteria: 1) was changing cross-stream direction (*i.e.*, $V_y(t_0) = 0$); 2) the path deflection within the previous or subsequent time step represented a significant change in direction (*i.e.*, $abs|\Delta Y_{crab}(t_{-1} \text{ to } t_0)| > 0.75$ cm or $abs|\Delta Y_{crab}(t_0 \text{ to } t_1)| > 0.75$ cm); and 2) the crab was in the process of moving rather than stopping (*i.e.*, $abs|V_x(t_0)| > 2$ cm s^{-1}).

The height of the crab's antennules (H_a) was recorded and used to measure the vertical changes in crab body position over the course of a track. . For each 3DLIF set, the elevation of the antennules was estimated through visual inspection of the recorded frames. Each block of images, corresponding to a single volume scan of the plume (20 LIF elevations), was manually examined, and the image containing reflections from the mouth region was identified to determine the height of the antennules during that 0.2 s period.

Various vectors used to describe the motion of a crab while tracking a plume are particularly useful in evaluating the turning behavior associated with successful plume tracking (Figure 3.3). A vector can be drawn from the crab to the source (**R**) indicating

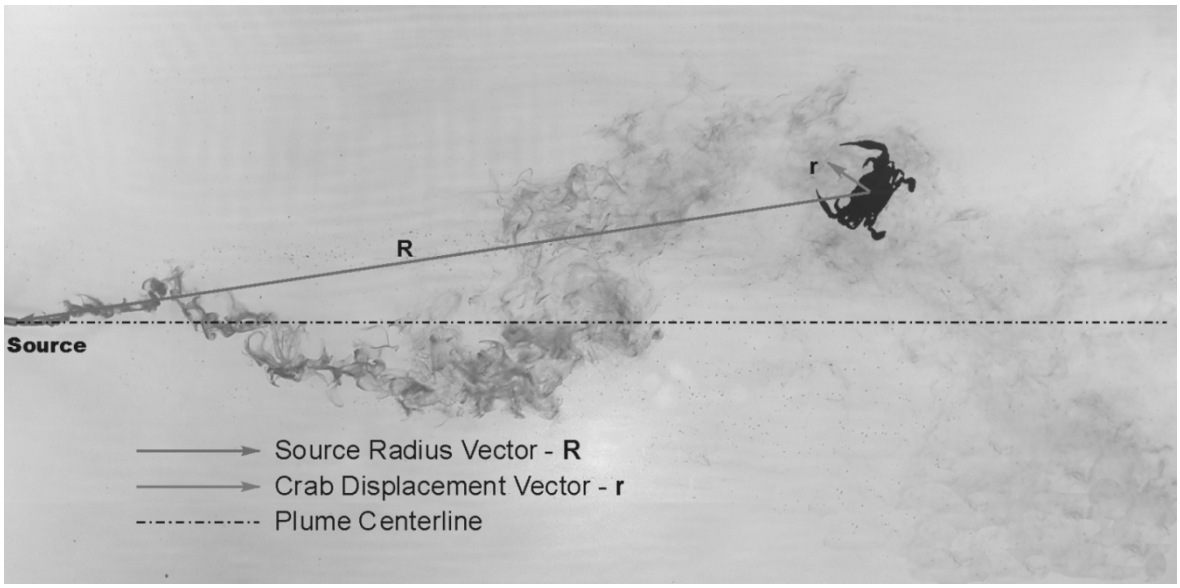

Figure 3.3. Characterization of vectors used for analysis of blue crab movement. The Source Radius Vector is drawn from the center of the crab directly to the source. The Crab Displacement Vector represents the magnitude and direction of movement of the crab in between two time steps. *Figure adapted with permission from Dickman (2008).*

what direction a crab would have to move to directly approach the source and how far away the crab is from the source. A displacement vector (**r**) indicates the direction and magnitude of a crab's movement between two time steps. The sign of this displacement vector is determined by the cross-stream (y) movement between two time steps in relation to the mean plume centerline (Figure 3.4).

Movement towards the mean plume centerline from either side of the line is considered an adjustment towards the source and is accordingly labeled as positive. Movement away from the mean plume centerline by tracking crabs results in a negative displacement vector. The resultant angle, α, from calculating a normalized dot product of

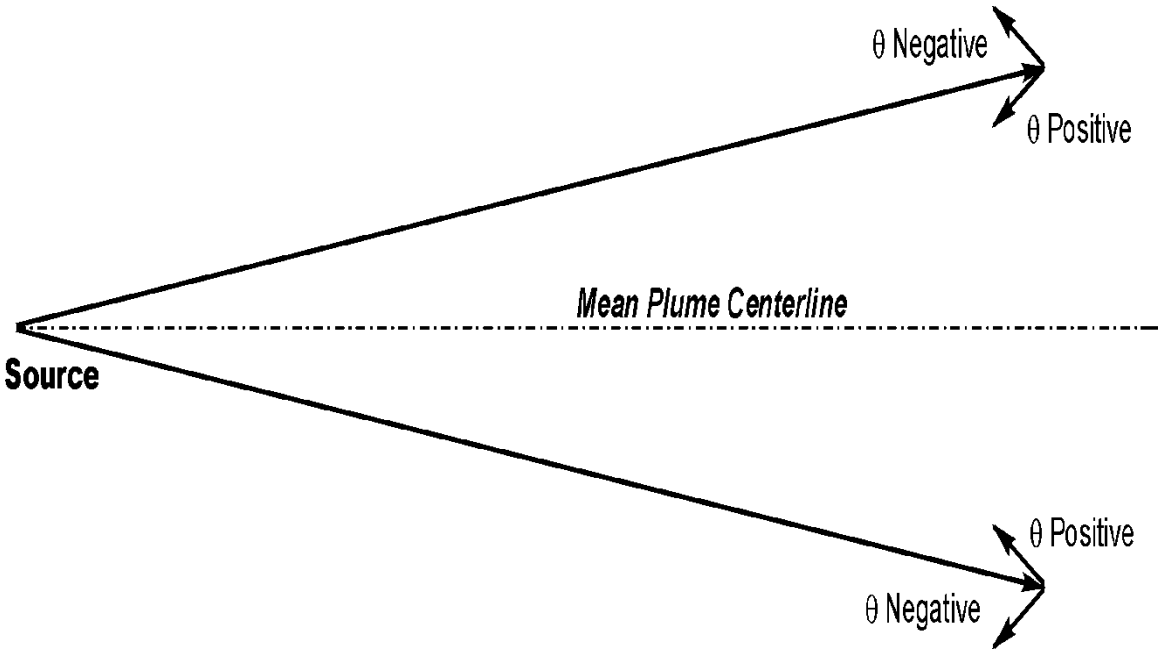

Figure 3.4. Diagram indicating angle sign criteria. Crab movement towards the mean plume centerline result in positive angles and movement away from the mean plume centerline result in negative angles. *Figure borrowed with permission from Dickman (2008).*

the source radius vector and the crab displacement vector at each time step (Eq. 3.1) provides a correlative measure of how directly a crab is moving towards the source.

$$\frac{\mathbf{r} \cdot \mathbf{R}}{|\mathbf{r}||\mathbf{R}|} = \cos \alpha$$

3.3.2 Concentration at the antennules

The resolution of our system was not such that we could determine each flick of the antennules and therefore we elected to create a sampling region in front of the antennules that encompassed the space that the antennules potentially could access. Figure 3.2 illustrates the location of this region and the methods used to calculate its location. At a point equally bisecting the distance between the LED's, a perpendicular

line was projected out towards the crab's mouthparts to create an axis on which to center the sampling volume. The distance from the LED axis to the crab's mouthparts along this line varied as a function of the size and shape of the crab. To automate the process of locating the antennule sampling region, the distance between the LED's and the mouth region was adjusted for each crab so the sampling region would fall just in front of the illuminated mouthparts. This distance remained the same for an individual crab throughout its track and ensured that no illumination from the mouthparts affected the concentration measurements in the sampling region. The size of this region (15 mm out from the mouthparts x 25 mm parallel to the mouthparts in one laser sheet) was chosen to represent the area that the antennules potentially access within one time step (~0.2 s) taking into account the advection due to flow within this time period, and the region was located at the appropriate elevation as described above. Both the average concentration across the patch (C_{Aavg}) and the maximum concentration within the patch (C_{Amax}) were determined, and used for later analysis.

3.3.3 Concentration at the legs

Data collection at the lower chemosensors was again inhibited as the shadows and the reflections cast by the crab's carapace, claws, and the walking legs themselves often obscured the plume structure near the leg chemosensors. For this reason, concentration arriving at the outer chemosensors was estimated by evaluating the structure of the approaching part of the plume that the chemosensors could potentially contact in the future. Once again, a sampling region was projected in front of the crab, far enough away to avoid shadow or reflection interference from the leading claw (Figure 3.5). This

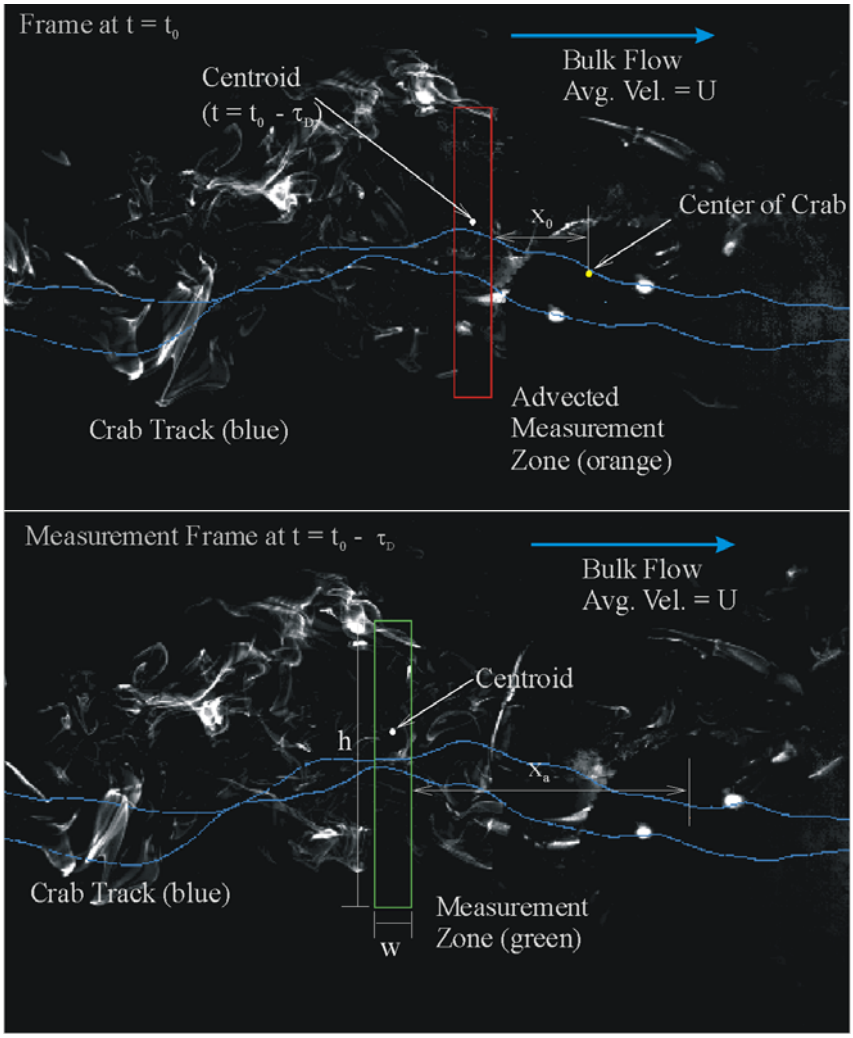

Figure 3.5. The location of the sampling zone for evaluating the signal at the walking appendage chemosensors. The panels depict an overhead view of a tracking crab at two points in time: (a) time $t = t_0$ and (b) time $t = t_0 - \tau_D$., where τ_D is the time delay between images. Due to shadowing concerns, concentration data are extracted at an earlier upstream location (green box in part b) and assumed to be advected to the chemosensory location (orange box in part a). Note that the bright spots in the lower portion of the orange measurement zone are crab legs and claws, which demonstrates the challenge of extracting accurate concentration data near the crab body. The desired sampling region position is initially located a distance upstream of the crab center position (X_0). To evaluate the sampling region location at an earlier time ($t_0 - \tau_D$), the sampling region is shifted upstream by the advection distance ($U\tau_D$). Hence, the distance from the crab center to the sampling zone (X_a) equals $U\tau_D + X_0 + \Delta X_{crab}$ (shown in upper image). The streamwise width of the sampling region, w, is determined by the advection distance $U\Delta t$ and the distance that the crab travels Δx between measurement samples, such that all chemosensory structure encountered by the crab between samples ($\Delta t \sim 0.21$ s) is included. The time delay (τ_D) shown corresponds to approximately 1 s. *Figure borrowed with permission from Dickman (2008).*

distance was matched to the advection distance and the crab's movement over a set time to determine at what point in time the sampling region would actually be available to the crab's chemosensors. Initial analysis revealed that the odor signal structure is largely unchanged over this short advection distance (Dickman 2008).

The size of the sampling region was based on the relative velocity of the crab (streamwise dimension) and the projected width of the crab (cross-stream dimension) (Figure 3.6). The streamwise dimension (*w*) needed to encompass the streamwise distance

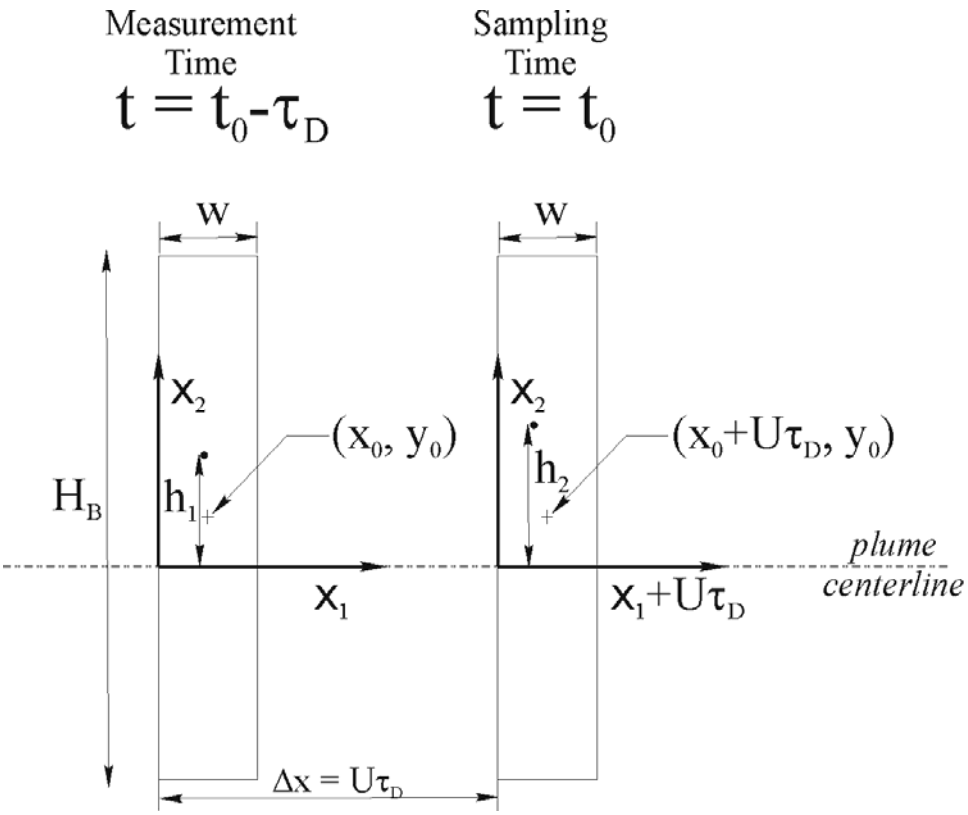

Figure 3.6. Details for the determination of the concentration centroid. h_1 and h_2 are the concentration centroids within the sampling volumes at the measurement ($t_0 - \tau_D$) and sampling t_0 times, respectively. *Figure borrowed with permission from Dickman (2008).*

covered by a crab within the sampling frequency (~0.2 s) and was determined by dividing the relative velocity of the crab (V_{rel}) by the sampling frequency (4.8 Hz). The cross-stream dimension (H_b) matched the projected cross-stream width of the crab and was manually calculated for each frame. The concentration measurements for these chemosensors were taken from the bottom laser plane, which was assumed to be the closest in proximity to the chemosensors themselves.

Concentration in this region was again analyzed for the maximum (C_{Lmax}) and the average (C_{Lavg}) concentration readings across the entire sampling plane. As we were interested in what the distribution of stimulus was across the region, we used a cross-stream concentration centroid (transverse coordinate = h) that represented the transverse location of the "center-of-mass" of the concentration distribution within the sampling region (Figure 3.6). This coordinate was compared with the transverse location of the center of the crab (Y_{crab}) to determine the deviation and a difference between these two values that was greater than ±0.5cm was used to indicate a transverse bias in the stimulus profile.

3.3.4 Statistical analyses and sample sizes

Most of the statistical analyses presented in Chapters 4-6 were performed in SYSTAT 12, for Windows (Version 12.00.08, SYSTAT Software, Inc., 2007). General linear model analyses were used for both univariate and multivariate tests of variance or covariance (F statistics with degrees of freedom and p values are reported). Analysis of categorical frequency data was performed by chi-square or log-linear, chi-square analysis using an online, interactive statistics tool provided by Vassar College and authored by Richard Lowry (VassarStats, 2008) (χ^2 and G^2 values with degrees of freedom and p

values are reported). Three-way statistical analyses on angles and related angular measures in this thesis were performed in MATLAB R2008b (Version 7.7.0.471, Mathworks, Inc., 2008) and utilized analysis of variance tests developed specifically for circular data as outlined in Zar (1998) and Harrison *et al.* (1986) (*F* statistics with degrees of freedom and *p* values are reported) with tables provided by Mardia (1972).

The analyses in Chapters 4-6 cover data obtained from crabs tracking in Continuous (N = 15 records), Meandering (N = 13 records), and Pulsed (N = 12 records) plumes. Repeated measures analyses covered two or three sections of the plume, thereby doubling or tripling the number of records for each plume type (Continuous = 30, 45; Meandering = 26, 39; Pulsed = 24, 36). Frequency analyses were based on records at each time step rather than means, and therefore sample size was increased dramatically as it was a function of the time spent tracking rather than the absolute number of tracks (Continuous = 862; Meandering = 1218; Pulsed = 927).

CHAPTER 4

GENERAL PATH CHARACTERISTICS FROM SIMULTANEOUS, 3DLIF MEASUREMENTS

4.1 Introduction

At the foundation of any investigation of tracking behavior are the basic features of the signal environment and path itself (*e.g.*, signal intensity or intermittency, search time, path linearity, velocity). This chapter lays the groundwork for the more in-depth investigation of causal factors driving blue crab search behavior by first summarizing these basic chemical stimulus and track features of crabs in Continuous, Meandering, and Pulsed chemical plumes, which were specifically derived by the 3DLIF measurement system. I made measurements to determine what constitutes a "signal" to tracking blue crabs, which is a critical piece of information for any of the analyses in this thesis that link behavior to chemical plume properties. Similarly, summarizing general characteristics of the paths themselves that crabs take to find the source sets the stage for determining what constitutes a "reaction" by a crab, and why different plume types elicit different tracking patterns.

4.2 Defining plume signal structure

4.2.1 Motivation

As discussed in Chapter 2, Section 4, the observation that blue crab walking speed decreases with increased bed roughness (*i.e.*, increased plume homogenization) suggests that large concentration spikes at the antennules mediate upstream motion. We see a

significant negative relationship between bed roughness and speed of tracking animals (Figure 2.1b), which mimics the negative relationship between bed roughness and plume homogeneity (as measured by the standard deviation of concentration fluctuations, Figure 5, Jackson *et al.* 2007) more closely than the negative relationship between bed roughness and intermittency (*see* Figs. 6 and 7, Jackson *et al.* 2007). These data suggests that it is the intensity of the bursts, rather than the frequency of bursts above background, that bears the strongest relationship to upstream movement. Because prior research has indicated that sensory adaptation renders neurons unresponsive to background stimuli (Atema 1985), it is likely that blue crabs employ a signal threshold that is based on signal contrast of well defined filaments, as opposed to absolute signal level. Obtaining a reasonable estimate of the signal threshold used by blue crabs is necessary before the responses to odor bursts can be further analyzed.

4.2.2 Concentration records

Concentration records were evaluated across all three plume types to determine the optimal way to define a "spike" in concentration at the crab's antennules. The greatest concentration within the two-dimensional antennule sampling box at each time point was normalized to the source concentration (C_0), resulting in a non-dimensionalized concentration maximum value ($C_{Amax} = C_{greatest}/C_0$). C_{Amax} values were subsequently averaged across the entire path of each track and also broken down according to distance from the source. Examining the mean of those C_{Amax} values in each plume reveals that C_{Amax} concentration is significantly affected by plume type as a whole ($F_{2,37} = 3.45$, $p = 0.042$; SYSTAT General Linear Models Analysis; Figure 4.1). Specifically, crabs in the

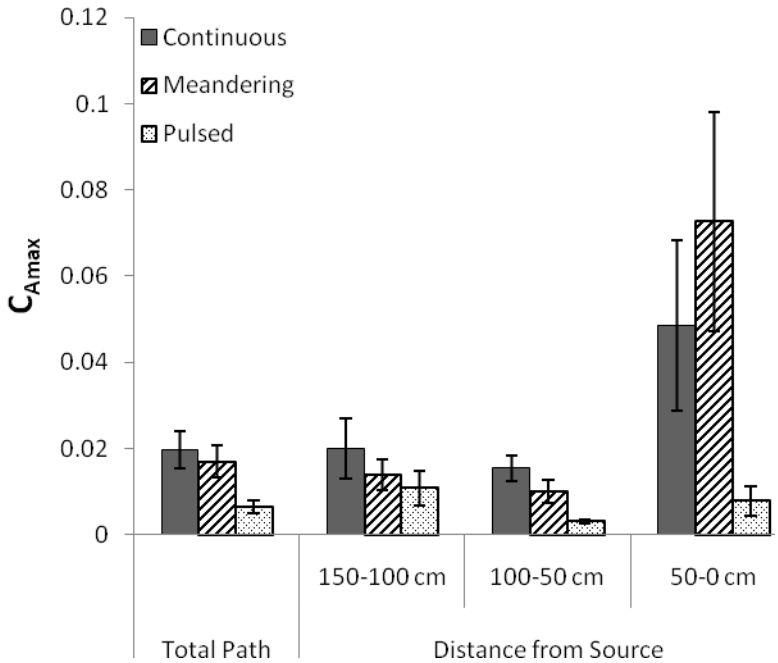

Figure 4.1. Mean of the average C_{Amax} value ± standard error of the mean (SEM) for individual crab tracks in various plume types. C_{Amax} is the observed maximal concentration in the antennule sampling region at a given time point normalized by the source concentration. The figure shows concentration maxima over the entire length of the track (Total Path) and for each segment of the track determined by distance downstream from the source (x = 150-100 cm, 100-50 cm, or 50-0 cm).

Pulsed plumes encountered filaments of statistically lower mean concentration than crabs in the Continuous and Meandering plume, which experience similar concentrations.

The analysis of how filament concentration changes as animals approach the source re-sampled individual crabs in different parts of the plume. To account for the re-sampling, I employed a repeat measures design to examine the effect of plume section. A repeat measures, 2-way ANOVA of plume type and distance from the source (divided into 3 increments: 150-100 cm, 100-50 cm, and 50-0 cm downstream of the source) indicates there is a marginally non-significant effect of plume type itself on the average value of C_{Amax} ($F_{2,34} = 2.87$, $p = 0.07$). There is a significant effect of plume section on average C_{Amax} concentrations encountered ($F_{2,68} = 8.5$, $p < 0.001$) and a significant

interaction between plume type and plume section ($F_{4,68} = 2.42$, $p = 0.05$). Crabs in the Pulsed plume encounter the lowest concentration filaments over the entirety of the track and experience concentrations that are 80% less , and much less variable, than the other plumes in the section closest to the source (50-0 cm downstream from the source). Crabs in the Continuous and Meandering plumes encounter the highest concentration filaments closest to the source and also experience the greatest spike variation in this section.

4.2.3 Exploring concentration thresholds

The substantial variation in stimulus concentration that crabs experience (across individuals, across plume types, and through time) complicates the task of determining a reasonable concentration threshold. Crabs are likely to respond to large concentration fluctuations (peaks) over a background of relatively low concentration so I performed an initial analysis to separate concentration "peaks" from the background. In this analysis, a concentration peak in a particular trial was defined crudely as every C_{Amax} point that was greater than the two C_{Amax} values surrounding it. It is unlikely that tracking crabs process all peaks as viable signals, therefore further analysis is necessary to determine what constitutes a signal to a tracking crab.

Due to the variability in concentrations encountered by crabs across plume types (Figure 4.1), it is unreasonable to use a fixed threshold to define which peaks are considered signals by tracking blue crabs. To account for this variability, a search for a reasonable spike concentration threshold focused on the relation of individual peaks to the average C_{Amax} concentration ($AvgC_{Amax}$) encountered by each crab during their entire track. Accordingly, I defined a "spike" as a occurring when the maximal concentration within the sampling region exceeded a set threshold defined relative to that animal's

$AvgC_{Amax}$ (Fig 4.2). Expressing the threshold as a function of the per crab average (C_{Amax}) accounts

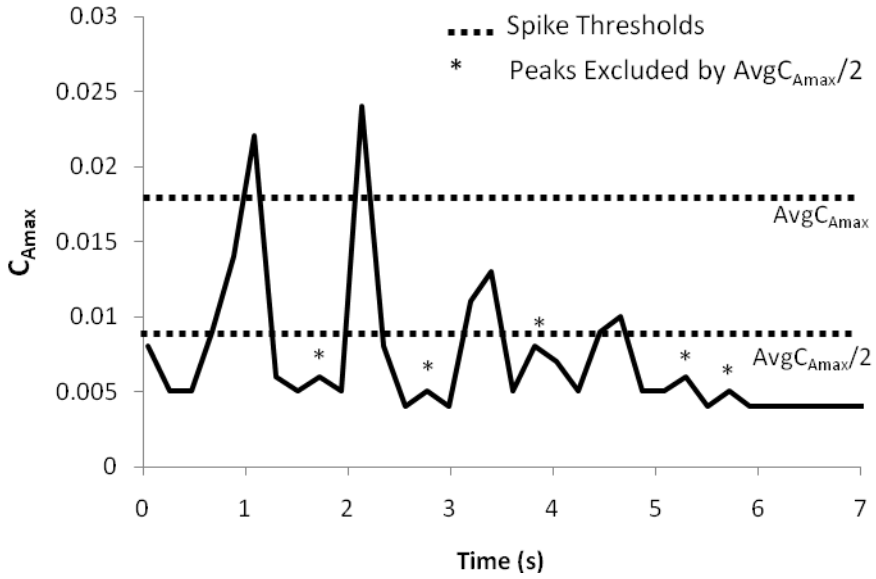

Figure 4.2. Representative C_{Amax} concentration record for a crab tracking in the Meandering plume with example $AvgC_{Amax}$ thresholds and excluded peaks marked.

for the variation experienced by individual crabs while still constituting a consistent criterion.

I examined the consequences of applying three threshold rules, $AvgC_{Amax}/4$, $AvgC_{Amax}/2$, and $AvgC_{Amax}$, by analyzing the number of potential peaks excluded by each threshold (Figure 4.3). As suggested, higher spike thresholds excluded a greater proportion of potential spikes. A spike threshold of one-fourth the average value of C_{Amax} ($AvgC_{Amax}/4$) (*i.e.*, peaks greater than $AvgC_{Amax}/4$ are considered spikes) excluded ~5-15% of all peaks and a spike threshold of one-half the average value of C_{Amax} ($AvgC_{Amax}/2$) excluded ~18-28% of all peaks. A spike threshold of the average value of C_{Amax} ($AvgC_{Amax}$) was by far the most exclusive, eliminating ~37-53% of all peaks that arrived at a crab's antennules. There was a significant effect of C_{Amax} threshold on the

percent of peaks excluded over the entire path length ($F_{2,111} = 34.25$, $p < 0.001$; SYSTAT GLM) but no significant effect of plume type ($F_{2,111} = 1.87$, $p = 0.16$) or interactive effect of plume type and threshold ($F_{4,111} = 0.61$, $p = 0.66$; Figure 4.4). This indicates that, as a first approximation, a standardized threshold over the entirety of the crab track captures most of the salient variation in C_{Amax} values, especially as average C_{Amax} values were computed individually for each crab.

A repeat measures analysis on the plume broken into downstream sections indicates that there is no significant interaction between plume type and plume section ($F_{4,156} = 1.78$, $p = 0.14$), no significant interaction between threshold and plume section ($F_{4,156} = 0.70$, $p = 0.59$), and no significant interaction between plume type, threshold, and plume section ($F_{8,156} = 0.55$, $p = 0.81$) on the percent of peaks excluded by our analyses (Figure 4.3). This serves to further justify the use of a standardized threshold as the percent of peaks eliminated is not significantly different over the individual sections of the plume for each plume type across all thresholds tested. Based on these analyses, we used a threshold of one-half the average C_{Amax} value ($AvgC_{Amax}/2$) in our subsequent analyses.

There is also a considerable amount of variability in the mean C_{Amax} values encountered by individual crabs within a single plume type. The variability in mean C_{Amax} concentrations experienced by tracking crabs in the Continuous plume alone extends over an order of magnitude (Figure 4.3). Therefore, we wanted to ensure our threshold was reasonable at the level of the individual crabs, particularly in relation to the average concentration and standard deviation of concentration fluctuations of the entire plume. Ideally, we would expect the peaks that we consider Spikes (*i.e.*, greater than our

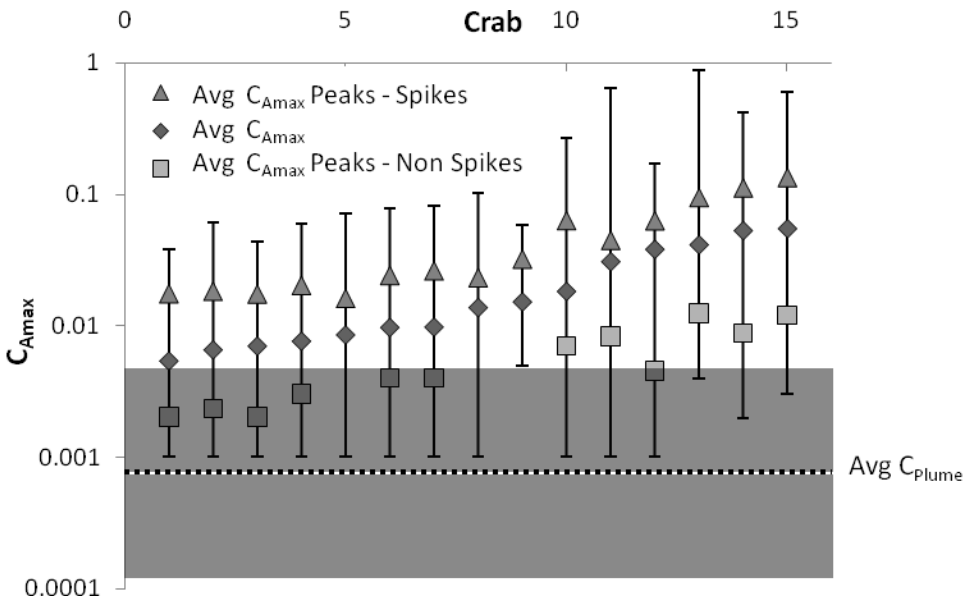

Figure 4.3. Average C_{Amax} values (♦) for individual crabs in the Continuous plume. Error bars represent the maximum C_{Amax} and minimum C_{Amax} values. Average peak C_{Amax} values are indicated in relation to a spike threshold of $AvgC_{Amax}/2$: Spikes (▲) are considered any peak that is greater than the threshold; Non Spikes (■) are the remaining peaks that are less than the threshold. Horizontal, dotted line represents the average concentration of the plume (C_{Plume}) at 4.5 cm above the bed taken from Dickman (2009), roughly corresponding with the height of the crab's antennules. Shaded region represents mean standard deviation of concentration fluctuations of the plume at 4.5 cm above the bed. Crab number (x-axis) was arbitrarily assigned to crabs in order of increasing average C_{Amax} value.

threshold) to be far above the base level of the standard deviation of concentration fluctuations (noise) present in a particular plume. Accordingly, we would also expect Non Spikes (*i.e.*, peaks below our threshold) to largely fall within the background noise of an individual plume. As mentioned previously, the $AvgC_{Amax}/4$ was the least exclusive of our thresholds and consequently failed to eliminate many peaks that fell within the noise level of the plume. While the $AvgC_{Amax}$ threshold would have been the most stringent, we chose to look at the $AvgC_{Amax}/2$ threshold to be more conservative about the peaks that we were eliminating as spikes. As seen in the Continuous plume (Figure 4.3), the average value of peaks which are greater than an $AvgC_{Amax}/2$ threshold

(Spikes) far exceeds the noise levels of the plume, while the average value of the peaks less than the $AvgC_{Amax}/2$ threshold (Non Spikes) fall within or very close to the average noise level of the plume. These findings further support our decision to use the $AvgC_{Amax}/2$ value as our spike threshold.

4.3 Path characteristics

4.3.1 Search time

The time crabs spend searching for the source (*i.e.*, overall period of trials) is significantly affected by plume type ($F_{2,37} = 5.50$, $p = 0.008$), with crabs in the Meandering and Pulsed plumes taking ~30-60% longer to traverse the entire test section than it takes crabs in the Continuous plume (Figure 4.4). The search time data suggest that

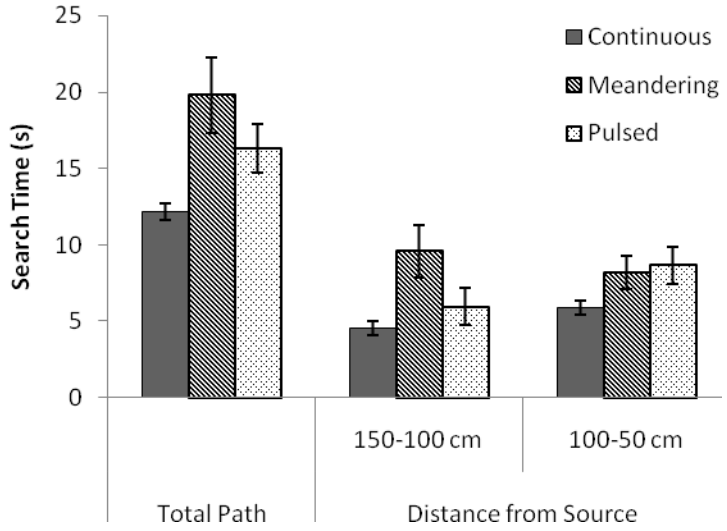

Figure 4.4. Mean search time (s) ± standard error of the mean (SEM) for crabs in various plume types. The figure shows data for crabs over the entire length of the track (Total Path = 150-40 cm downstream from the source) and for each segment of the track determined by distance downstream from the source (x = 150-100 cm or 100-50 cm).

tracking a Pulsed plume may be of intermediate difficulty between tracking a Continuous or Meandering plume due to its intermediate search time.

Search time also was analyzed using a repeat measures, 2-way ANOVA, with plume type and plume section as main effects. Due to constraints of the system outlined in the methods section (*see* Chapter 3, Section 2.3), records of crab and plume data did not cover the entire upstream section of the plume. Consequently, our estimates of the time spent in the upstream section of the plume (*i.e.*, 50-40 cm from the source) are omitted from the present section analysis, as they are not representative of true search time in this section. The analysis reveals a significant effect of plume type on the time it takes for crabs to navigate the downstream and middle sections of the plume ($F_{2,37}$ = 4.48, p = 0.018). Crabs in the Continuous plume take less time to traverse a particular section than crabs in either the Meandering or Pulsed plumes, which is consistent with the lowest total search times displayed by crabs in the Continuous plume (Figure 4.4). There is not a significant effect of plume section ($F_{1,37}$ = 1.68, p = 0.20) but there is a marginally significant interactive effect of plume type and plume section ($F_{2,37}$ = 3.04, p = 0.06) on per section search time. Data indicate that crabs in the Continuous and Pulsed plumes take longer to traverse the middle section of the plume (100-50 cm) than they take in the downstream section (150-100 cm), whereas crabs in the Meandering plume spend equal amounts of their search time in the downstream and middle sections of the plume.

4.3.2 Stopping

Crabs sometimes stopped while tracking (*i.e.*, total velocity between -0.5 cm s^{-1} and 0.5 cm s^{-1} sustained for greater than 0.5 s) and the percent of time crabs spent stopped differed over plume type and plume section (Figure 4.5). Because the percent of

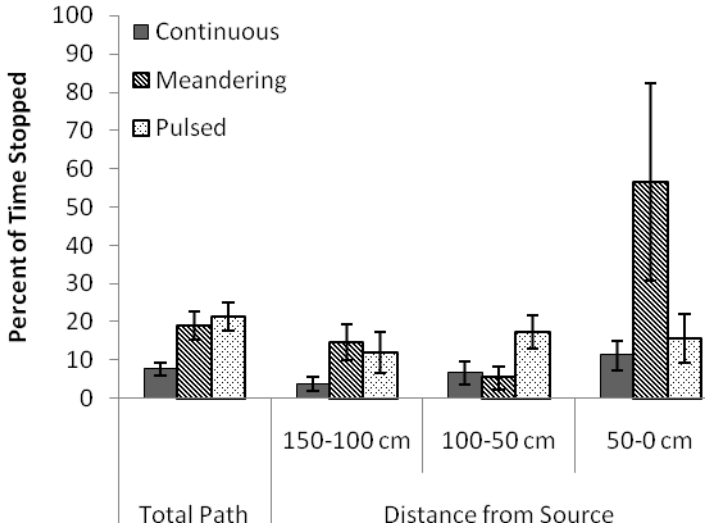

Figure 4.5. Mean percent of search time that crabs spend stopped ± standard error of the mean (SEM) for crabs in various plume types.

time stopped is not a function of the absolute time spent in a section, we can justifiably incorporate data from all three sections in this particular analysis. Crabs in the Meandering and Pulsed plumes spent a significantly greater percent of their track time stopped than crabs in the Continuous plume by a two- to three-fold increase ($F_{2,37} = 5.94$, $p = 0.006$; SYSTAT GLM; Figure 4.5). A repeat measures ANOVA on percent of time stopped as a function of section indicates that there is a significant effect of plume section on the percent of search time stopped ($F_{2,68} = 3.69$, $p = 0.03$), with the greatest percent of search time stopped closest to the source. There is nearly a significant interaction effect between plume type and plume section on the percent of search time stopped ($F_{4,68}$ =

2.49, $p = 0.05$). Crabs in the Continuous plume spend a progressively greater percent of search time stopped as they move upstream towards the source, whereas the percent of track time stopped does not appear to change in relation to plume section for crabs in the Pulsed plume.

The average number of stops that a crab made was also significantly different among the different plume types for the entire plume length ($F_{2,37} = 3.83$, $p = 0.03$; Figure 4.6). On average, crabs in the Meandering plume made twice as many stops as crabs

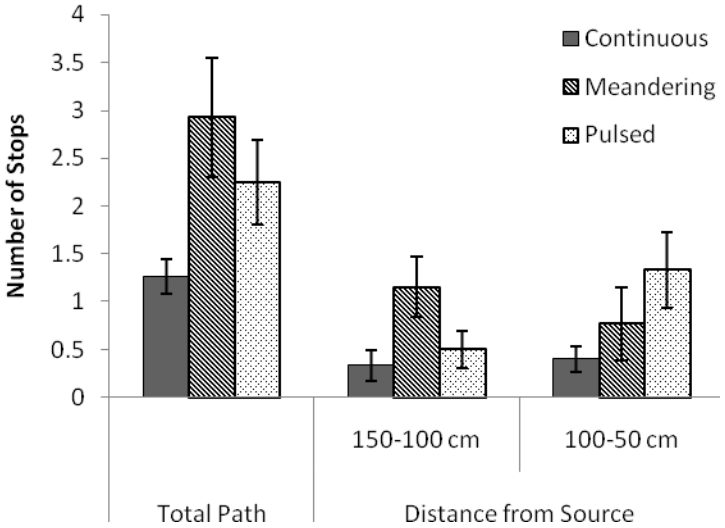

Figure 4.6. Mean number of times individual crabs stopped while tracking ± standard error of the mean (SEM) for crabs in various plume types.

in the Continuous plume. Crabs in the Pulsed plume made an intermediate number of stops over the trial length, which more closely approached the number of stops made by crabs in the Meandering plume than the stops made by crabs in the Continuous plume. A repeat measures ANOVA on the average number of stops as a function of section indicates that there is no significant effect of section on the number of stops crabs made

during their track ($F_{1,37} = 0.91$, $p = 0.35$). However, there is a significant plume type and plume section interaction on the number of stops made by tracking crabs ($F_{2,37} = 3.66$, $p = 0.04$). Crabs in the Continuous and Meandering plumes make a similar number of stops in the downstream and middle sections of the plume, while crabs in the Pulsed plume appear to increase the number of stops they make from the downstream to the middle section of the plume.

The period of those individual stops was not significantly different across plume types ($F_{2,81} = 2.38$, $p = 0.1$; Figure 4.7) and there is also no significant interaction effect

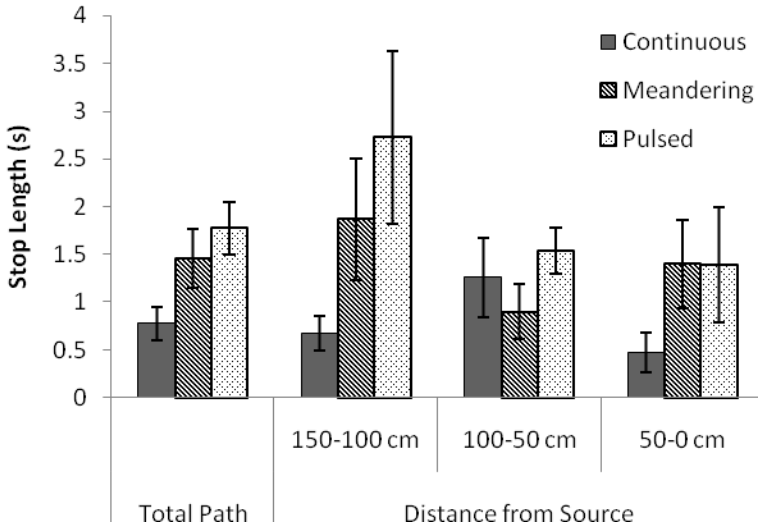

Figure 4.7. Mean time period of each stop ± standard error of the mean (SEM) for crabs in various plume types.

between plume type and plume section on the length of individual stops ($F_{4,34} = 0.54$, $p = 0.70$).

4.4 Tracking speed

4.4.1 Total velocity

Examining the mean total velocity incorporates both the cross-stream and along-steam velocity components and therefore gives us a measure of the overall motion of a tracking crab. The mean total velocity of tracking crabs is not significantly affected by plume type ($F_{2,37} = 1.15$, $p = 0.33$; Figure 4.8), although animals in Continuous and Pulsed

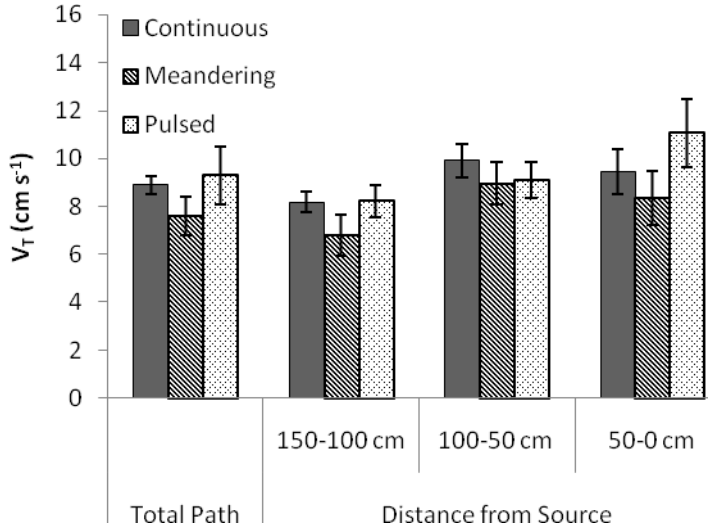

Figure 4.8. Total velocity ± standard error of the mean (SEM) for crabs in various plume types.

plumes display similar velocities, and are both higher than the Meandering plume. A 2-way, repeat measures ANOVA of plume type and section determined that the mean total velocity of crabs is significantly affected by plume section ($F_{2,72} = 5.73$, $p = 0.005$) but not by the interaction between plume type and section ($F_{4,72} = 0.94$, $p = 0.44$).

Although mean velocities are not affected significantly by plume type, the significant effect of plume on velocity distribution (Figure 4.9; $\chi^2 = 190.56$, $df = 18$, $p < 0.001$) indicates that crabs in the Continuous plume tend to move at higher velocities than

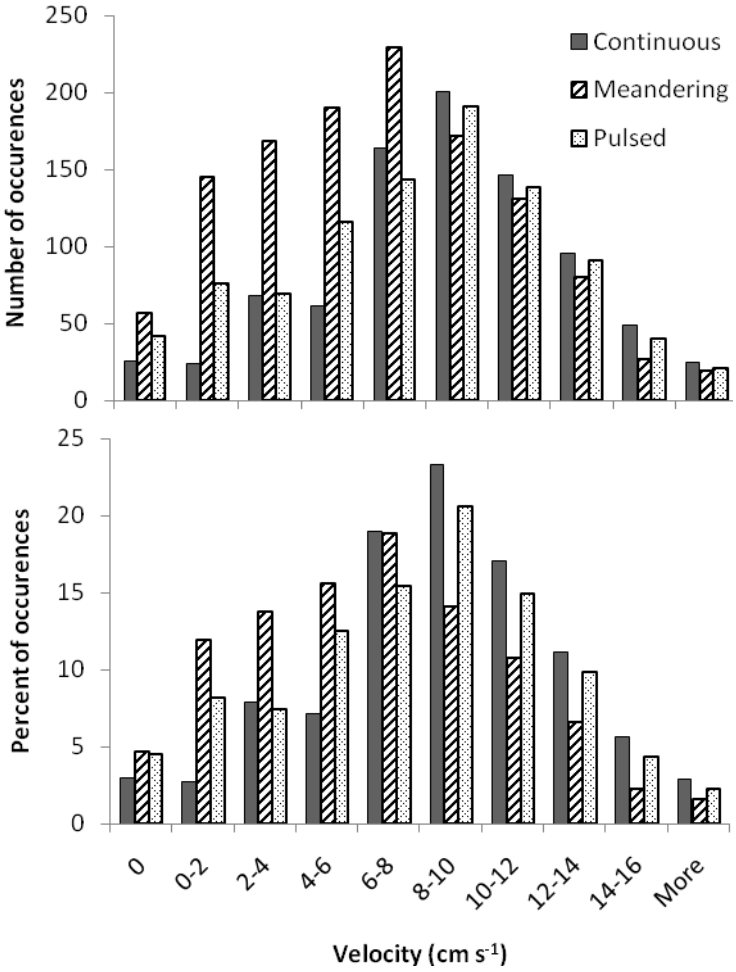

Figure 4.9. (a) Frequency and (b) percent distribution of crab's total velocity across various plume types.

do crabs in the Meandering or Pulsed plumes. As suggested by the location of the peak velocity, movement speed for the Meandering plume is skewed towards slower velocities, while the total velocity distribution of the Pulsed plume more closely mimics the velocity distribution of crabs in the Continuous plume, both of which display a higher percentage of greater velocities.

Crabs in the downstream section (150-100 cm from the source) of the Continuous and Pulsed plumes have similar total velocity distributions, and they are clearly skewed towards greater total velocities than the distribution for crabs in the Meandering plume (Figure 4.10). This effect of plume type on the total velocity distribution in the downstream

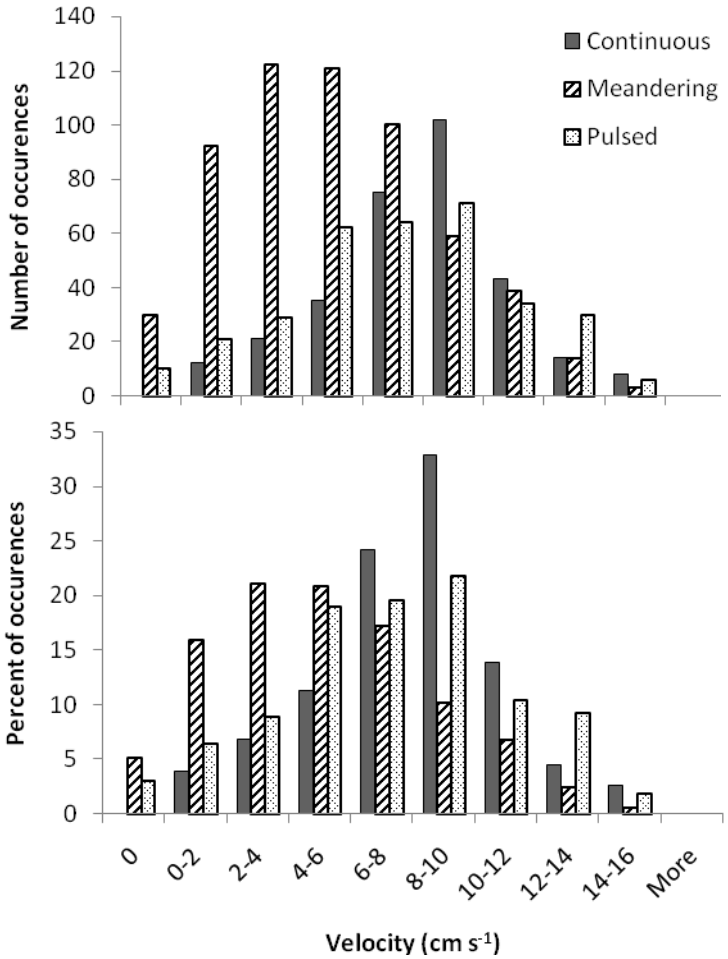

Figure 4.10. (a) Frequency and (b) percent distribution of crab's total velocity in the downstream section (150-100 cm from the source) of various plume types.

section is significant ($\chi^2 = 199.12$, $df = 16$, $p < 0.001$).

The frequency distributions of total velocity of different plume types are still significantly different in the middle section of the plume (Figure 4.11; $\chi^2 = 65.11$, $df = 18$, $p < 0.001$). The crabs in the Continuous plume retain a bias towards faster total velocities than crabs in either of the other plumes. The total velocity distributions of crabs in the

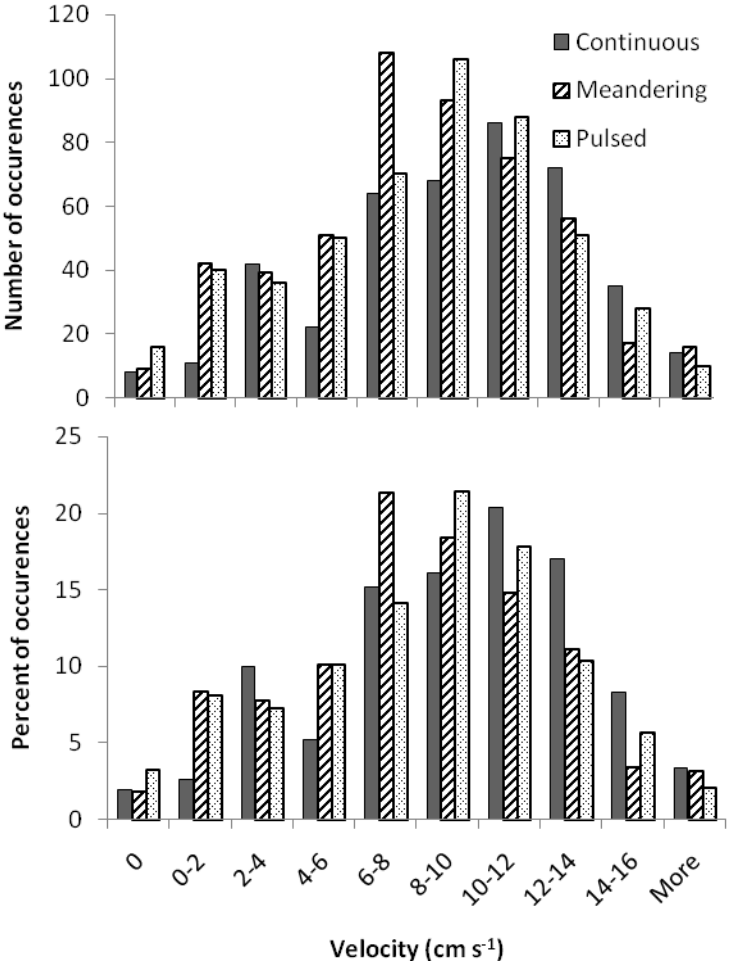

Figure 4.11. (a) Frequency and (b) percent distribution of crab's total velocity in the middle section (100-50 cm from the source) of various plume types.

Pulsed and Meandering plumes are more skewed towards lower velocities than the Continuous plume distribution; both have a small, local frequency peak at very low (< 2 cm s^{-1}) velocities.

In the upstream section of the plume, the total velocity distribution for the Pulsed plume again seems to be an intermediary between the Continuous and Meandering plumes (Figure 4.12) and plume type has a significant effect on velocity distribution ($\chi^2 = 41.73$, $df = 18$, $p = 0.001$). Crabs in this section of the plume spend a great deal of time stopped regardless of plume type but also demonstrate higher velocities than in the

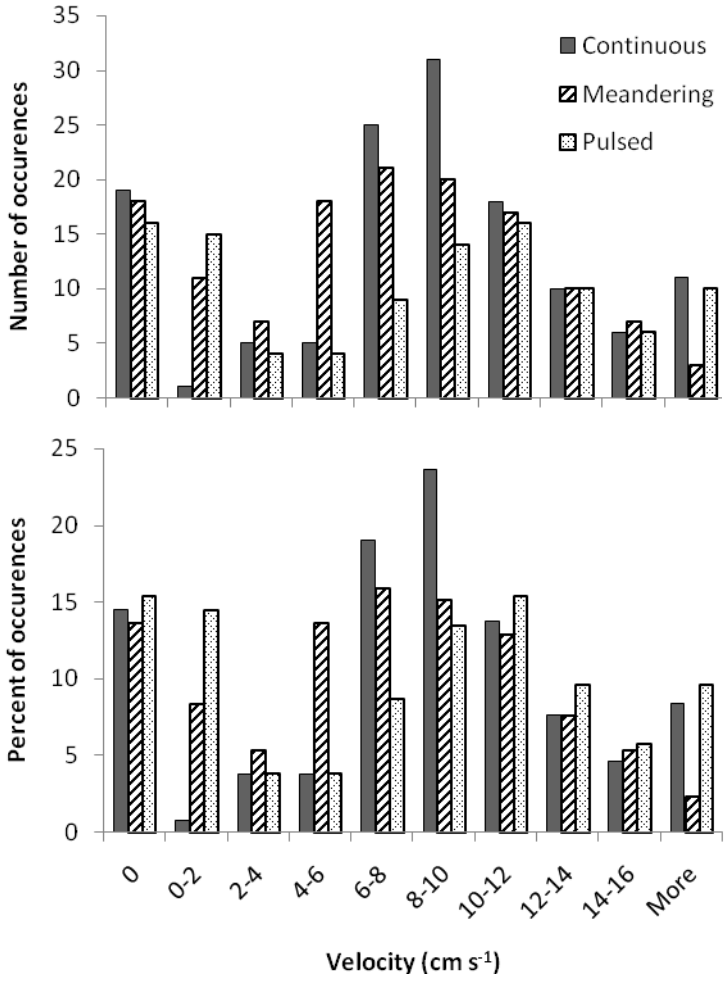

Figure 4.12. (a) Frequency and (b) percent distribution of crab's total velocity in the upstream section (50-0 cm from the source) of various plume types.

previous plume sections, creating strong, bimodal velocity distributions. The Meandering total velocity distribution is skewed towards slower velocities but still

reaches relatively high velocities (> 10 cm s^{-1}) more frequently in this section than in the sections further downstream. Crabs in the Pulsed plume have a total velocity distribution that has equally large frequency peaks at 0 cm s^{-1} and at 12 cm s^{-1}, but the distribution is skewed towards faster velocities.

4.4.2 Along-stream velocity

Mean total velocity gives a good indication of overall crab movement while tracking, but only the along-stream component indicates actual progression towards the upstream source location. Therefore, I decided to break total velocity into its components to measure the progress of crabs towards the source and ultimately examine the effects of plume structure on a crab's progress towards the source. When total velocity is broken down into along-stream (x-direction) and cross-stream (y-direction) components, the mean along-stream velocity of crabs across the entire length of the test section is significantly affected by plume type ($F_{2,37} = 5.18$, $p = 0.01$; Figure 4.13). This seems

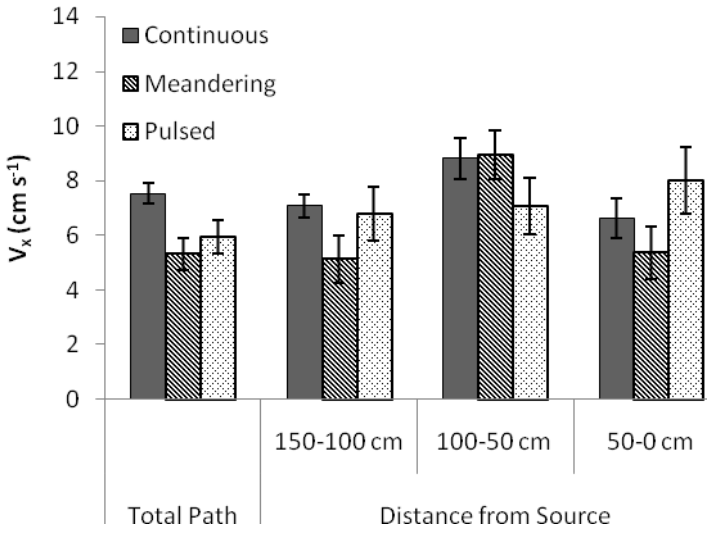

Figure 4.13. Mean along-stream (x) velocity ± standard error of the mean (SEM) for crabs in various plume types.

to suggest that the lack of significant effects of plume type on mean total velocity (*i.e.*, Figure 4.8) indicates differences in transverse versus upstream movement. Crabs in the Continuous plume move upstream towards the source more quickly than crabs in the Meandering and Pulsed plumes by ~2 cm s^{-1} on average. As before, the velocities of crabs in the Meandering plume are intermediate between the Continuous and Pulsed plumes.

Plume type has a significant effect on the along-stream velocity distributions (χ^2 = 313.56, df = 18, $p < 0.001$). The along-stream velocity distributions depict a bimodal distribution in the Pulsed plume and, to some extent, in the Meandering plume (Figure 4.14), whereas the velocity distribution in the Continuous plume is unimodal. The bimodal distribution in both plumes has a local minima at 6 cm s^{-1}, which is also roughly the mean along-stream velocity for both plumes. Crabs in the Meandering plume have a strong tendency to move along-stream at low velocities and stop along-stream motion. The along-stream velocity of the Pulsed plume appears to be an intermediate distribution between the Meandering and Continuous plumes with the bimodal peaks of the Pulsed plume aligning with the major peak in each of the other plumes. This continues the trend of the Pulsed plume being an intermediary between the two other plumes that we have repeatedly seen in search time (Figure 4.4), number of stops (Figure 4.6), and their velocity distribution in the upstream section (Figure 4.12).

4.4.3 Upstream and downstream velocities

As indicated by the frequency distribution (Figure 4.14), mean patterns in along-stream velocity may reflect plume specific differences in upstream and downstream movement (*i.e.*, towards the source and away from the source, respectively). Thus, I

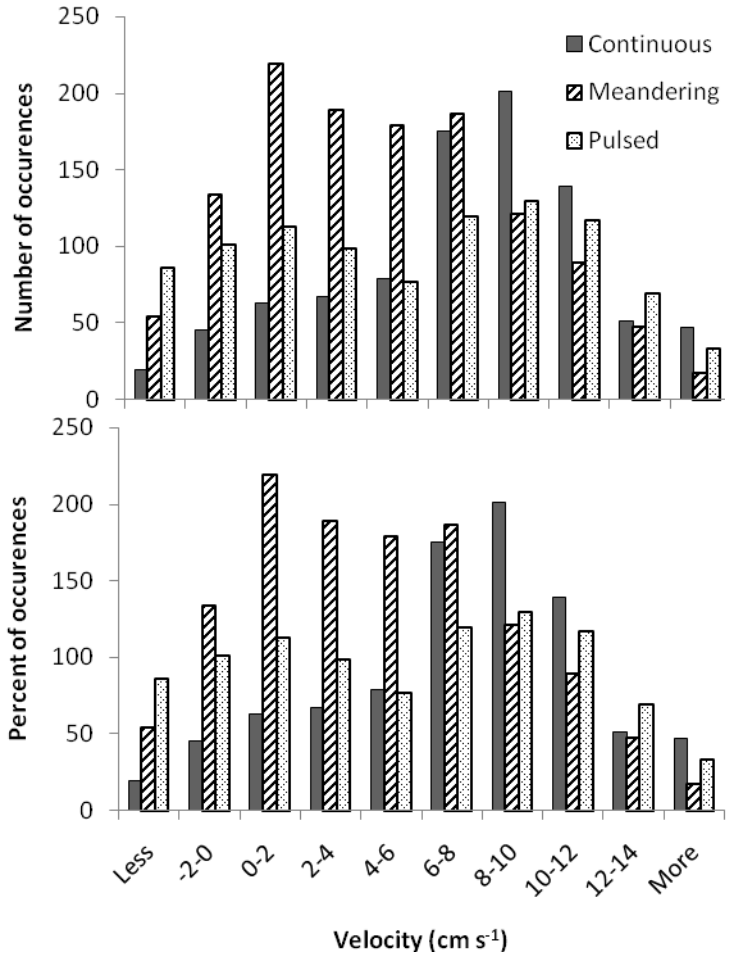

Figure 4.14. (a) Frequency and (b) percent distribution of crab's along-stream (x) velocity in various plume types.

elected to analyze further only the upstream samples, which denotes progress to the source. Similar to the analysis of total velocity, there is a marginally significant effect of plume type on the mean upstream component of crab's along-stream velocity over the entire plume ($F_{2,37} = 2.86$, $p = 0.07$; Figure 4.15). As in the previous analysis, crabs in the Continuous plume move upstream the quickest and crabs in the Meandering plume move upstream the slowest. There is a significant effect of plume section on a crab's upstream velocity ($F_{2,72} = 7.43$, $p = 0.001$). There is a parallel pattern between in the previous analysis, crabs in the Continuous plume move upstream the quickest and crabs

in the overall along-stream velocity (Figure 4.13) and the upstream component of the along-stream velocity (Figure 4.15) indicating that crabs spend most of their time moving forward.

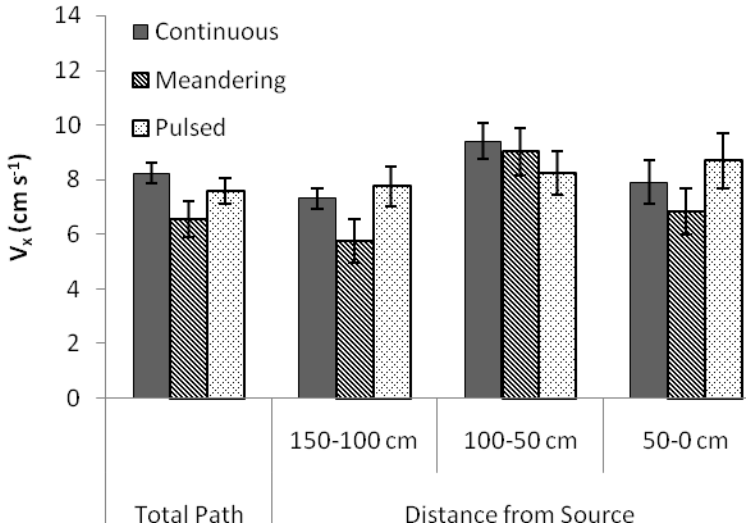

Figure 4.15. Mean upstream (positive x) velocity ± standard error of the mean (SEM) for crabs in various plume types.

Crabs in the Continuous and Meandering plumes moved towards the source fastest in the middle section of the plume, while crabs in the Pulsed plume showed consistent movement across all plume sections. There was no significant interaction effect between plume type and plume section on the upstream velocity of tracking crabs ($F_{4,72} = 1.55$, $p = 0.20$).

Downstream crab velocity for the total path was not significantly different across plume types ($F_{2,34} = 0.29$, $p = 0.75$; Figure 4.16). Although velocities do seem to differ in various downstream locations, downstream crab velocity by parts cannot be analyzed by a repeated measures ANOVA since individual crabs rarely went downstream more than once. Crabs in the Continuous plume tended to move downstream progressively faster as they approached the source. In the upstream section of the plume, crabs in the

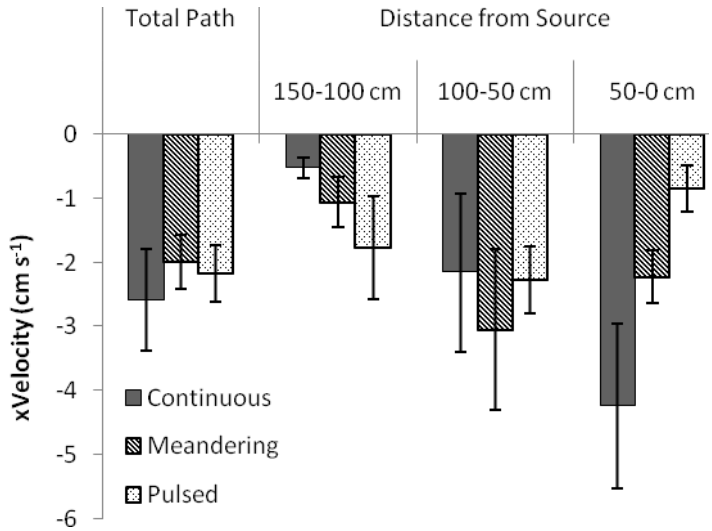

Figure 4.16. Mean downstream (negative x) velocity ± standard error of the mean (SEM) for crabs in various plume types.

Continuous plume went downstream faster than the crabs in the Meandering and Pulsed plumes and crabs in the Pulsed plume moved downstream the slowest.

As suggested above, downstream movements were infrequent, and crabs spent on average less than 20% of their total track time traveling downstream (Figure 4.17). Crabs

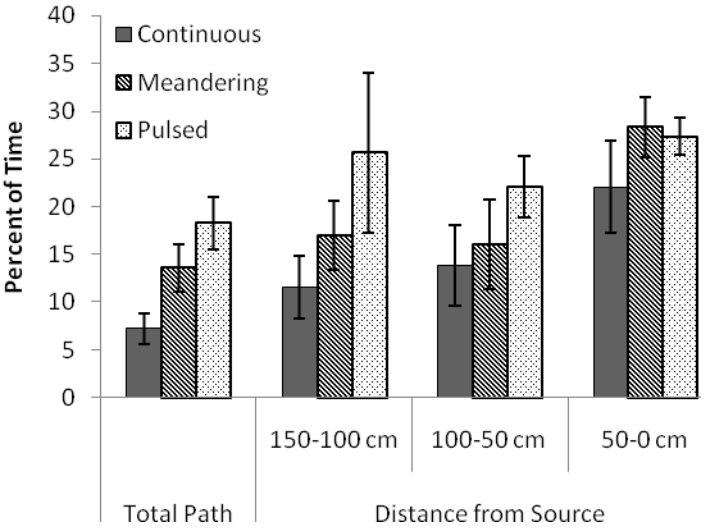

Figure 4.17. Mean percent of search time spent traveling downstream (negative x) ± standard error of the mean (SEM) for crabs in various plume types.

in the Continuous plume spent the lowest percent of their search time traveling downstream, followed by crabs in the Meandering and Pulsed plumes. Crabs in the Continuous plume spent an increasing percent of their search time moving downstream as they got closer to the source (Figure 4.17); crabs additionally moved downstream at the fastest velocities in the upstream section of the Continuous plume (Figure 4.16). Crabs in the Meandering plume spent the greatest percent of time traveling downstream closest to the source and an equal percent of time traveling downstream in the other two plume sections (Figure 4.17). Crabs in the Pulsed plume traveled downstream for an equal percent of time in each of the sections.

4.5 Net to gross displacement ratio

As mentioned in Chapter 2, Section 2.3, net to gross displacement ratio (NGDR) is a measure of path linearity, where an NGDR of 1 would indicate a completely straight path and lower values would indicate increasingly indirect trajectories. NGDR typically is used to indicate whether animals move directly towards the source or in more circuitous routes, and I examined this metric to determine if crabs seemed to locate sources more easily in particular plume types (Figure 4.18).

Plume type had a signficant effect on the NGDR of tracking crabs over the total path ($F_{2,35} = 7.57$, $p = 0.002$, values were arc sine transformed to meet normality criterion for statistical analysis), with crabs in the Continuous plume traveling in straighter paths than crabs in the Meandering or Pulsed plumes, which holds Crabs tend to increasingly straighten their path as they approach the source, as demonstrated by the significant effect of plume section on NGDR ($F_{2,68} = 15.53$, $p < 0.001$). However, there is not a significant interactive effect between plume type and plume section on NGDR ($F_{4,68} = 0.30$, $p =$

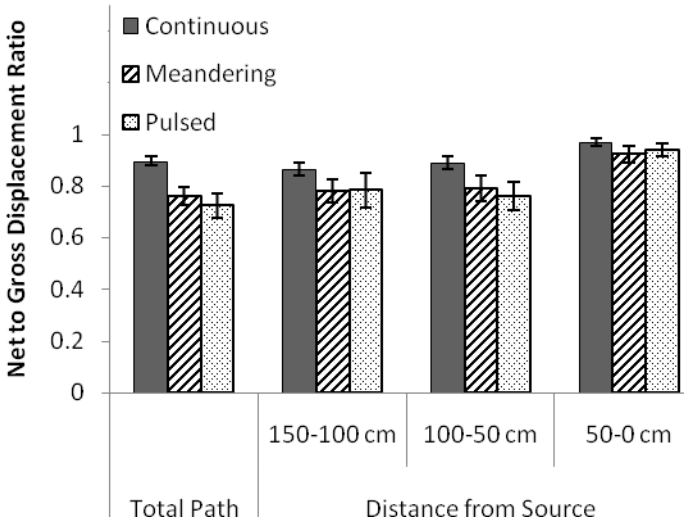

Figure 4.18. Mean net to gross displacement ratio (NGDR) ± standard error of the mean (SEM) for crabs in various plume types.

0.87), indicating that the same general NGDR relationship exists between plume types in each plume section.

4.6 Antennule height

Previous studies have examined the tracking behavior of crabs in two dimensions, along-stream and cross-stream. In addition to these measurements, the 3DLIF system was able to record crab antennule height (*see* Chapter 3, Section 3.1, for details of method), adding a third dimension to our examination of crab tracking behavior. Because crabs are so adept at tracking a plume in two-dimensions, I hypothesized that crabs would be able to follow specific plume properties in three-dimensions, raising and lowering their body in response to changes in concentration characteristics.

Following release, odorant plumes experience turbulent mixing as they are advected downstream, thereby causing the plume to spread in the horizontal and vertical directions with increased distance from the source. It appears that crabs in the plume with the least amount of turbulent mixing (*i.e.*, Continuous plume; Figure 4.19) keep their

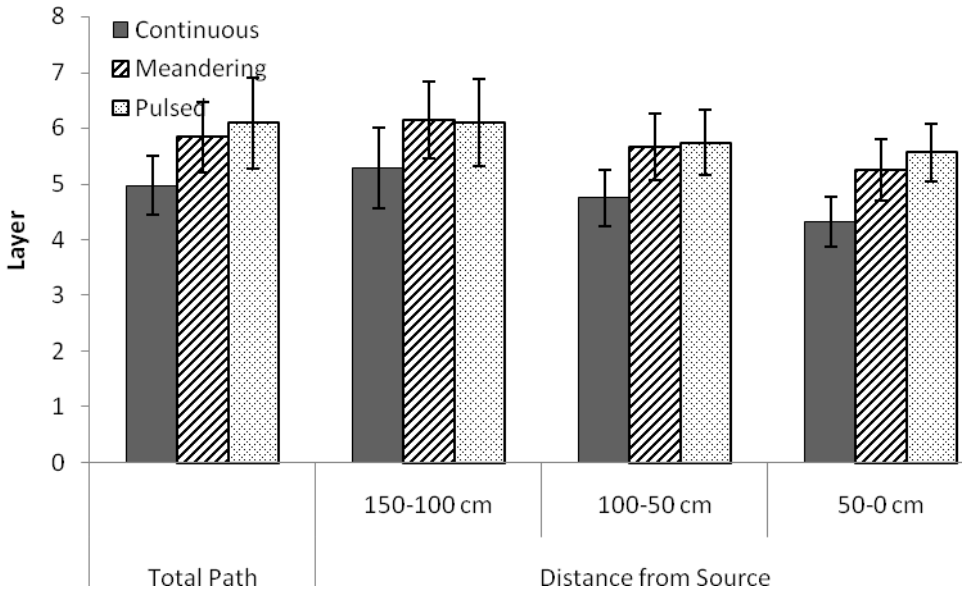

Figure 4.19. Mean antennule height ± standard error of the mean (SEM) for crabs in various plume types.

antennules lower than crabs in the plumes with greater turbulent mixing (*i.e.*, Meandering and Pulsed plumes), supporting the idea that crabs are vertically following specific plume properties with their antennules. While this effect of plume type is not significant ($F_{2,45}$ = 0.76, p = 0.47), a repeat measures analysis indicates there is a highly significant effect of plume section on antennule height ($F_{2,90}$ = 7.35, p = 0.002). Crabs clearly lower the height of their antennules as they move closer to the source, and this trend holds true across all plume types as there is no significant interaction between plume type and plume section ($F_{4,90}$ = 0.36, p = 0.84). This supports the hypothesis that crabs are changing the height of their antennules in response to plume properties.

4.7 General results summary

The analyses in this chapter have led us to a definition of a concentration "spike", constituting an odorant "signal" for blue crabs, and defined basic path characteristics, within which we can now characterize crab reactions to odorant signals. Dickman (2008)

performed complementary experiments to address the characteristics of the specific plume types used in this study, but focused on mean statistical properties of odor plumes, rather than defining the signals actually impinging on crabs as they track the source. Combining the signal and behavior data of the crab tracks with data about the plume structure will help establish the importance of particular aspects of chemical signals during navigation.

4.7.1 Spike definition and concentration information

To make any meaningful measurements of crab tracking behavior in response to plume properties, I first needed to define what constitutes an odorant signal to a tracking crab by examining odorant concentration arriving at the crab's antennules (C_{Amax}). The threshold measure that I derived (*i.e.*, $AvgC_{Amax}/2$) defines antennule concentration peaks as an actual signal spike when they are well above the mean plume concentration. The mean value of peaks that are not considered spikes often falls within the standard deviation of the mean plume concentration (*i.e.*, $AvgC_{Plume}$) and generally falls above this deviation for crabs that experience mean C_{Amax} concentrations at their antennules greater than two standard deviations from the mean C_{Plume} concentration.

The fundamental concentration measurements that helped form the spike definition demonstrated that crabs tracking in the Continuous and Meandering plumes encounter considerably more concentrated C_{Amax} values in the upstream section of the plume than the mean concentration they encounter over the total path (Continuous: mean = 0.02, upstream = 0.049; Meandering: mean = 0.017, upstream = 0.073). These C_{Amax} measurements also go through wider concentration fluctuations than the mean (Continuous: mean standard error (SE) = 0.004, upstream SE = 0.02; Meandering: mean

SE = 0.004, upstream SE = 0.025). While these C_{Amax} measurements were specific to concentration at the crab's antennules, this tendency towards greater plume dilution farther away from the source is characteristic of all plumes, and particularly plumes with isokinetic release (Dickman 2009). In contrast, the Pulsed plume was introduced as a jet (*i.e.*, with momentum compared to the surrounding fluid), causing more immediate dilution and homogenization, which is reflected in the less variable C_{Amax} concentrations encountered by crabs tracking in the upstream section in this condition (Figure 4.1).

4.7.2 Plume difficulty

The time that it takes successful searchers to traverse the test section and the related number of the stops made by tracking crabs can be considered measures of the difficulty in tracking individual plume types. The significantly different search times employed by crabs across various plume types (Continuous: ~12 s; Meandering: ~20 s; Pulsed: ~16 s) confirms our initial assumption that Meandering and Pulsed plumes are more challenging for blue crabs to successfully track than Continuous release plumes (Figure 4.4) as a result of their greater signal intermittency and/or lateral spread. Data on the stopping characteristics of each path also support this observation. Crabs in the Meandering and Pulsed plumes spent a significantly greater amount of their time stopped (Figure 4.5) and made more stops (Figure 4.6) than crabs in the Continuous plume. Crabs in the Pulsed plumes displayed search times (Figure 4.4) and number of stops (Figure 4.6) that were intermediate to the Continuous and Meandering plumes. This adds weight to the argument that the Pulsed plume represents a tracking problem of intermediate difficulty compared to the other two plume types.

These basic data still do not tell us what particular plume properties are causing the observed differences in locomotory behavior. However, findings from Dickman (2008) regarding plume structure leads to some hypotheses about what might be causing the differences in overall track time and stop time across the different plumes, and the "intermediate" behavior of crabs in the Pulsed plume. Dickman (2008) found that close to the source in the Continuous plume, high levels of intermittency (*i.e.*, greater consistency of plume contact) were a good indicator of coincidence with the plume centerline. This is also the case for the Pulsed plume but there we see that the average concentration is roughly half that of the Continuous plume. In the Meandering plume, high values of intermittency occur at greater distances from the centerline due to cross-stream mixing. Thus, intermittency may be a poor indicator of coincidence with the plume center under these conditions. Average concentration in the Meandering plume is also reduced by an order of magnitude compared to the Continuous plume. These data suggest that a combination of intermittency and plume concentration may be an indicator of the level of difficulty that a plume presents to a tracking crab. This seems to be borne out by the fact that crabs in the Meandering plume have the longest search times (Figure 4.4) as they must deal with decreased concentration and intermittency that is not coincident with the plume centerline. Crabs in the Pulsed plume only experience decreased average concentrations and they have an intermediate search time compared to the two other plumes. Finally, crabs in the Continuous plume take the shortest time to find the odorant source and have the benefits of coincident plume centerline with intermittency and the greatest average plume concentrations.

4.7.3 Total tracking velocity

The mean total velocity of tracking crabs appears to be similar across all plume types (Figure 4.8) although crabs vary in the length of time they take to traverse the entire plume and individual sections within the plume (Figure 4.4). Because mean total velocity incorporates time stopped as well as along-stream and transverse movement, which may be positive or negative, this measure of velocity reflects both upstream surges, putatively mediated by the detection of odor filaments, as well as transverse movements that may be in response to transverse signal contrast. Therefore, examining the measure of total mean velocity is limited in its ability to provide insight to the specific plume characteristic inducing behavior differences. The frequency distribution of the total velocity (Figure 4.9) is only slightly more informative. The frequency distribution among plume types within the 0 cm s^{-1} bin (*i.e.*, instances of crabs stopping) appropriately mimics the distribution of average number of stops between plume types over the total path (Figure 4.6). The general skew of each plume frequency distribution (Figure 4.9) indicates that crabs in the Meandering plume tend towards lower velocities (< 10 cm s^{-1}) and crabs in the Continuous plume tend towards higher velocities (> 10 cm s^{-1}). This also correlates to the search time data (Figure 4.4) indicating crabs in the Continuous and Meandering plumes traverse the test section in the least and greatest amount of time, respectively. The frequency distribution of the Pulsed distribution (Figure 4.9) very closely matches the frequency distribution of the Continuous plume at higher velocities (> 10 cm s^{-1}) but tends towards lower velocities with greater frequency than the Continuous plume distribution. As mentioned earlier (Section 4.6.2), these tracking features may be a direct result of the concentration and intermittent arrival of

individual filaments. A high intermittency factor that is coincident with the plume centerline combined with high average concentrations (Continuous plume) appears to be the easiest to track; high intermittency coincident with centerline but with low average concentrations (Pulsed plume) seems to be of intermediate difficulty; and high intermittency non-coincident with the centerline and low mean concentration (Meandering plume) may be the most difficult for crabs to successfully track.

A bimodal distribution of velocity is observed when the total velocity frequency distributions are examined by flume section, particularly for crabs in the Meandering and Pulsed plumes (Figure 4.11). This bimodal pattern is very strong for all plume types in the most upstream section (Figure 4.12) and there is a slight suggestion of this trend in the middle plume section for Meandering and Pulsed plumes. These bimodal patterns are indicative of a surging and stopping behavior, which may be due to repeated antennule signal acquisition and loss. Both Meandering and Pulsed plumes consist of variation on larger time and space scales (Dickman 2008), thus creating longer/larger gaps between scented and unscented fluid compared to the Continuous plume. Crabs appear sensitive to periods of odor absence on these larger scales (Keller and Weissburg 2004), which may be leading to the greater degree of bimodality close to the source and the weak bimodality displayed by crabs in the middle sections (100-50 cm downstream from the source) of Meandering and Pulsed plumes.

4.7.4 Along-stream velocity

Partitioning the total velocity up into along- and upstream components provides a more informative analysis of crab responses, since along-stream velocity is a direct indication of a crab's progress towards the source. This data reveals that crabs in the

Continuous plume are, on average, making the most rapid progress towards the source (Figs. 4.13, 4.14) and the magnitude of the along-stream velocity is damped in the Meandering and Pulsed conditions. This damping in the Pulsed plume is largely a factor of the bimodal along-stream velocity distribution, again indicative of a stopping and surging behavior of crabs along the entire path. The distribution of along-stream velocities in the Meandering plume (Figure 4.14) is strongly biased towards slower velocities (< 10 cm s^{-1}) but shows a slight bimodal tendency. This tendency is indicative of the same slowing and surging towards the source that we see in the Pulsed plume. However, because we did not see this pattern in the total velocity distribution of the Meandering plume (Figure 4.9) it is likely that the periods of intermediate or decreased velocity towards the source are concurrent with increased transverse movement.

4.7.5 Downstream tracking velocity

Plume type has no effect on the downstream component of along-stream velocity (Figure 4.17). We do not have the opportunity to compare the significance of plume section between the two velocity measures as multicolinearity issues prevent analysis of the downstream data (Figure 4.17) by section. However, it is interesting to note that the downstream pattern of movement by section indicates that, when crabs in the middle section of the plume move downstream, they do so at similar speeds. The patterns of downstream velocity in the sections farthest away from and closest to the source are mirror images of each other. In the downstream section, crabs in the Continuous plume move downstream relatively slowly when they do move downstream and crabs in the Pulsed plume move relatively quickly downstream. In the upstream section of the plume, crabs in the Continuous plume move downstream rapidly whereas crabs in the Pulsed

plume move downstream relatively slowly. These strongly inverted relationships eliminate plume differences in downstream velocity when they are averaged, indicating that analyses on the total path downstream velocity data must be interpreted cautiously.

4.7.6 Upstream tracking velocity

The upstream motion of crabs dominates the along-stream velocity and accordingly we see a similar, plume dependent pattern between the two data sets. Crabs in the Continuous plume have the greatest along-stream (Figure 4.15) and upstream motion (Figure 4.16) overall. Crabs tracking in the Continuous and Meandering plumes progress towards the source fastest in the middle section of the plume (Figure 4.16), indicating that the middle section of the plume is easiest for them to track. Conversely, crabs in the Pulsed plume maintain the same velocity over the entirety of their track. The signal intensity encountered by crabs in these plumes decreases with increasing distance from source and the plume width increases with increasing distance from the source (Dickman 2008). We also know that the Pulsed plume initially is homogenized by the jet introduction and it keeps a relatively constant and narrow width throughout the test section. Keller *et al.* (2003) demonstrated that concentrated filaments arriving at the antennule chemosensors play a major role in motion towards the source, therefore the crabs should have an easier time tracking the closer they are to the source. Data also suggest that crabs use their leg chemosensors to help them orient in the plume and appear to use some form of comparison across chemosensors to accomplish this task. Perception of the "edge" of chemical plumes by sensing low/no-odor at chemosensors on one side of the body and high odor on the other side is aided by the plume being an intermediate width. A narrow plume decreases the number of chemosensors that will detect a signal

regardless of its concentration, whereas a wide, homogenous plume makes edge detection difficult and concentration gradients are more subtle. It appears then that the combination of signal concentration and plume width defines the upstream speed of tracking crabs. Crabs in the Continuous and Meandering plumes would experience elevated concentration closer to the source and the width of the plume is likely to be 'optimal' for tracking in the middle section of the plume. The crabs in the Pulsed plume, which do not appear to experience much difference in concentration or plume width over the entire track, support this hypothesis as their speed remains relatively constant over the entire track.

4.7.7 Maintaining cross-stream plume contact

The greater width of the Meandering plume, due to the intensified turbulent transport from the upstream cylinder, indicates that crabs in these plumes would demonstrate increased transverse movement to stay in contact with the plume, which is corroborated by the NGDR data (Figure4.18). Crabs in the Meandering and Pulsed plumes have depressed NGDR values compared to crabs in the Continuous plume, which indicates they arrived at the source by more indirect paths. NGDR can be decreased in this manner as a result of backwards movement and cross-stream movement. The pronounced bimodal pattern in the overall along-stream velocity of crabs in the Pulsed plume (Figure 4.14) that is similar to that seen in the total velocity near the source (Figure 4.12), indicates that the bimodal distribution is truly caused by the crab slowing (even going backwards) and surging towards the source. Farther away from the source, when the overall along-stream velocity distribution (Figure 4.14) does not match the total velocity distributions (Figs. 4.10 and 4.11) as closely, it is more likely that the

intermediate along-stream velocities are concurrent with increased cross-stream movement.

As would be expected due to the differences in plume width and meander, animals in the Continuous plume move in significantly more direct paths to the source than animals in the other two conditions (Figure 4.18). It is somewhat surprising that crabs in the Meandering and Pulsed plumes have identical NGDR's. The increased plume width would initially suggest that crabs tracking the Meandering plume would have the greatest cross-stream motion and therefore should have a lower mean NGDR than crabs in both the Continuous and Pulsed plumes. However, analysis suggests that crabs in the Pulsed plume spent a significantly greater percent of their track time moving downstream compared to crabs in the other plumes (Figure 4.17). The NGDR of a path can be increased by movement away from the source in a downstream direction in addition to cross-stream movements so it appears that the increased percent of time that crabs in the Pulsed plume spend going downstream had a significant effect on the NGDR of their path. Bed roughness experiments (Chapter 2) indicated that blue crabs increase transverse movement (lower NGDR; Figure 2.1a) in response to plume intermittency caused by increased turbulence intensity. The introduced meander and pulses in the odorant plumes alter the spatial and temporal intermittency of the plume, and therefore it seems clear that this intermittency is affecting the NGDR of crabs in the Meandering and Pulsed plumes. What is less clear is what aspects of this intermittency cause crabs to adopt indirect trajectories and, in particular, what are the differences between the two plume types that cause crabs in the Meandering plume to increase transverse movement while crabs in the Pulsed plume increase downstream movement.

4.7.8 Maintaining vertical plume contact

A crab's ability to move transversely to maintain contact with the plume (Figure 4.18) is complemented by its ability to move vertically to maintain contact with the plume (Figure 4.19). Because the height of plume increases as it is advected away from the source, crabs that begin tracking the plume with their antennules near the upper edge of the plume would lose plume contact as they approach the source and the plume boundaries become lower. This tracking problem is similar to the issue crabs would have if they simply moved upstream in response to an odorant stimulus; unless the crabs were already along the plume centerline, they would eventually pass the odorant source as the plume narrows approaching the source. It is perhaps not surprising then, yet still remarkable, that crabs possess this ability to "steer" vertically within the plume just as they are able to steer in the cross-stream direction.

Vertical antennule placement within the plume is a novel behavior that has not previously been described in the literature, largely due to inadequate technology. For this reason, any qualitative description of the behavior itself is an important contribution to our understanding of blue crab tracking behavior; however, the quantitative measurements of vertical behavior combined with the odorant signal structure made possible by the 3DLIF system has the potential to significantly advance our knowledge of tracking as a whole. In particular, understanding the cues that affect antennule placement in the third-dimension may help explain anomalies we see in the two-dimensional response patterns of blue crabs to particular stimuli.

CHAPTER 5

ALONG-STREAM MOTION RESULTS FROM SIMULTANEOUS, 3DLIF MEASUREMENTS

5.1 Introduction

The studies on crab tracking in turbulent boundary layers (Chapter 2) relate detailed concentration data from a series of increasingly turbulent plumes with behavior from crabs tracking similar, but separate, plumes. These studies show that crabs track more slowly in homogenized, highly turbulent plumes than do crabs in filamentous plumes, indicating that filamentous plume structure is an important factor mediating upstream motion. This is supported by general observations on path kinematics from the 3D laser experiments (Chapter 4). Crabs exhibit longer search times in plumes with greater levels of mixing (Figure 4.1). Crabs in these plumes also stop with greater frequency (Figure 4.6), stop for longer periods of time (Figure 4.7), and have slower along-stream velocities (Figure 4.13). The high variability of individual filament concentration along the length of a path and between individual searches (Figs. 4.2 and 4.3), suggests that it is the relative concentration of individual filaments above background levels, rather than the absolute concentration or frequency of filament encounter, that stimulates upstream motion. Our data support the hypothesis that reception of concentration spikes at the crab's antennules stimulate a crab to move forward and loss of those signals cause a crab to slow down or stop forward motion (Keller *et al.* 2003; Jackson *et al.* 2007).

The results presented in this chapter examine forward motion of blue crabs during tracking to test hypotheses linking upstream motion to plume properties. Here, I attempt to correlate simultaneous plume and behavior data from blue crabs tracking in three different plume types, Continuous, Meandering, and Pulsed, more closely than has been previously possible. The three source release types enabled spatial and temporal comparisons of plume structure with resulting forward motion. The Meandering plume was used to test the effects of a wider plume with large-scale spatial intermittency, specifically creating a plume where high levels of intermittency and strong concentration was not necessarily coincident with the plume centerline. The Pulsed plume was used to test the effects of large-scale temporal intermittency on crab tracking behavior, and the Continuous plume provided a baseline to which to compare the behavior of crabs in the other plume types. Perhaps not surprisingly, these data reveal a much more complex picture of how blue crabs utilize chemo- and mechanosensory signals to mediate their search behavior than simply relying on reception of concentration bursts at the antennules. There is evidence that signals at the leg chemosensors play a significant role in mediating upstream motion and indication that crabs sometimes slow down or stop in response to concentration bursts at the antennules.

5.2 Spike encounter while tracking

Analyzing the properties of chemical stimuli that reach crab antennules is a logical first step to answer the question of what signals mediate movement towards the source for tracking blue crabs. In Chapter 4, these stimulus records were mined to establish criteria to define concentration spikes above background concentration. I determined that a normalized, rather than fixed, threshold was most appropriate to define

spikes and found that a threshold based on the average C_{Amax} value of individual crabs (specifically $AvgC_{Amax}/2$) provided a reasonably stringent, yet not exclusive, spike cutoff. I first used this working definition of a signal to analyze the stimulus records in order to define patterns of stimuli arriving at the antennules. Due to the high variability of spike concentrations between paths and within paths (Figure 4.3), it is unlikely that absolute concentrations govern blue crab behavior as they move towards the source. Connecting dynamic patterns of stimuli arriving at the antennules to behavior provides a reasonable alternative to analyzing absolute concentration.

The time between spikes arriving at the antennules (interspike interval) differs as a function of plume type ($F_{2,18} = 4.06$, $p = 0.04$; SYSTAT GLM with plume type and section analyzed in a repeat measures design; Figure 5.1) and downstream distance

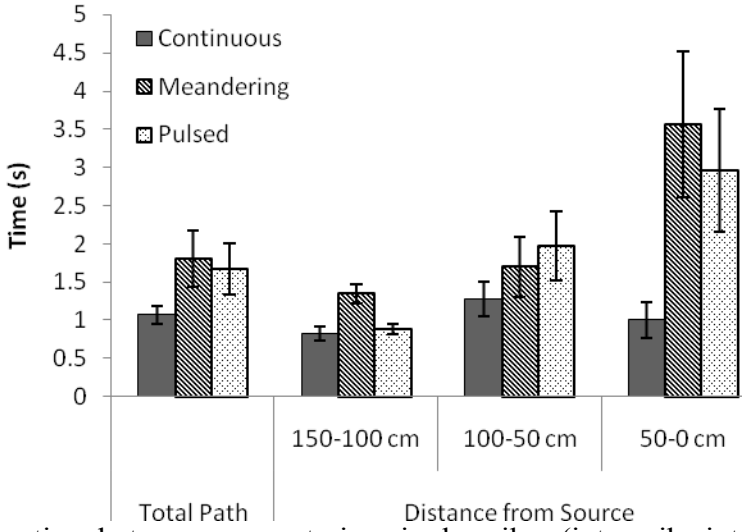

Figure 5.1. Mean time between encountering single spikes (interspike interval) ± standard error of the mean (SEM) for crabs in various plume types. The figure shows data for crabs over the entire length of the track (Total Path) and for each segment of the track determined by distance downstream from the source (x = 150-100 cm, 100-50 cm, or 50-0 cm downstream of the source).

from the source (plume section; $F_{2,36} = 8.86$, $p = 0.001$). The length of the interspike interval is also significantly affected by the interaction between plume type and plume section ($F_{4,36} = 2.83$, $p = 0.04$).

In general, the interspike intervals for crabs in the Continuous plume are shorter than those experienced by crabs in the Meandering or Pulsed plumes. The magnitude of this effect is contingent on distance downstream from the source as interspike intervals generally increase with decreasing distance from the source. Farthest downstream, crabs in the Meandering plume experience 50% longer interspike intervals (~1.5 s) than crabs in the Continuous and Pulsed plumes, which have similar interspike intervals (~1 s). Crabs in Meandering and Pulsed plumes experience intermediate, yet similar interspike intervals in the middle section of the plume whereas crabs in the Continuous plume experience their longest interspike intervals in this section. Time between spikes increases dramatically (50-100 %) as crabs traverse the upstream section of the Meandering and Pulsed plumes relative to intervals farther from the source. These interspike intervals are two- to three-times greater than intervals experienced by crabs in the Continuous plume, where intervals exhibit relative constancy with distance downstream from the source.

5.3 Spike encounter preceding acceleration

I examined the coupling between upstream movement and stimulus arrival at the antennules by computing the percentage of events where upstream accelerations are preceded by a concentration spike. The previous analysis of interspike intervals (Figure 5.1) indicates that spikes reach crab antennules on the order of one every 1-2 seconds. In accordance with the hypothesis that greater frequency of antennule spikes should produce

acceleration events, it is expected that the great majority of acceleration events in the crab tracks would be preceded by an antennule spike within one second. A preliminary analysis of how often a crab's acceleration is preceded within one second by a concentration spike at its antennules surprisingly revealed that approximately 60 % of crabs in the Continuous plume received a spike at their antennule in that time period before accelerating (Figure 5.2). Crabs in the Meandering and Pulsed plumes only

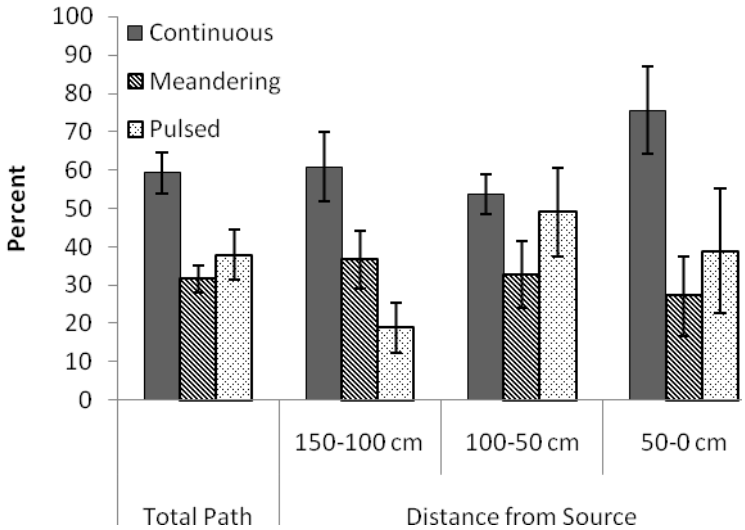

Figure 5.2. Mean percent of times acceleration is preceded within one second by a concentration spike at the antennules ± standard error of the mean (SEM) for crabs in various plume types.

received a spike at their antennules within one second before accelerating in 30-40 % of the acceleration cases. This effect of plume type on spike reception is significant ($F_{2,37}$ = 8.07, p = 0.001); however, a repeat measures analysis indicates that there is no significant effect of plume section itself on the percent of times an acceleration is preceded within one second by an antennule spike ($F_{2,58}$ = 0.26, p = 0.77), nor an interactive effect between plume type and plume section ($F_{4,58}$ = 1.53, p = 0.21).

By expanding the analysis window and looking at the percent of instances where crabs received spikes at their antennules within two seconds prior to accelerating, the percentage in the Continuous plume is increased up to roughly 85 % and up to 55-60 % for crabs in the Meandering and Pulsed plumes (Figure 5.3). Thus, prior concentration

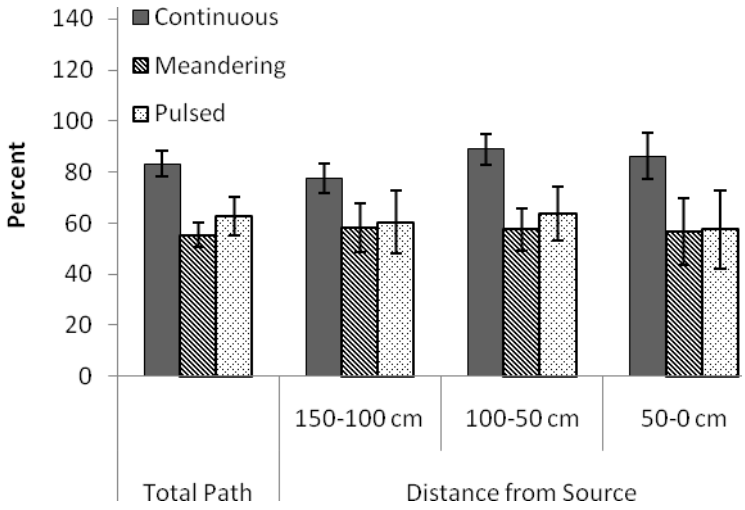

Figure 5.3. Mean percent of times acceleration is preceded within two seconds by a concentration spike at the antennules ± standard error of the mean (SEM) for crabs in various plume types.

spikes strongly predict acceleration but do not invariably lead to acceleration. This effect of plume type remains significant for the total path ($F_{2,37} = 6.63$, $p = 0.004$). There still is not a signficant effect of plume section on the percent of times that a crab's acceleration is preceeded within two seconds by an antennular concentration spike ($F_{2,58} = 0.09$, $p = 0.91$), and the interactive effect between plume type and plume section remains significant ($F_{4,58} = 0.21$, $p = 0.93$).

5.4 Average velocity relative to spike encounter

5.4.1 Average velocity after receiving an antennule spike

There may be a link between the frequency of spike reception and a crab's velocity as crabs in the Continuous plume experience shorter intervals between spikes (Figure 5.1) and take the least amount of time to traverse the test section compared to crabs in the Meandering and Continuous plumes. Spike and velocity records were examined to determine lag times between stimulus arrival and the subsequent animal response. As before, stimuli above the threshold of $AvgC_{Amax}/2$ were defined as a spike. Specifically, I examined the time that it took for a crab to reach above-average velocity after receiving a concentration spike at the antennules within a two-second window following a spike (Figure 5.4). It is important to note that this analysis does not take into

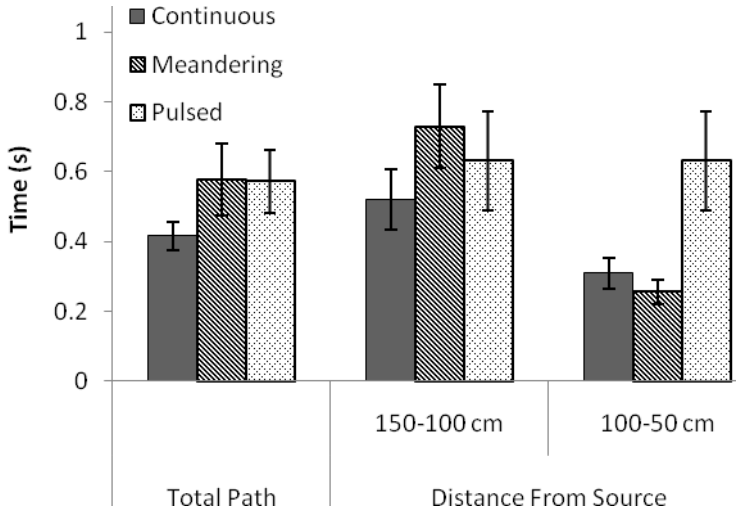

Figure 5.4. Mean time that it takes crabs to reach above-average velocity following an antennule spike ± standard error of the mean (SEM) for crabs in various plume types. **Note that there were not enough data points in the 50-0 cm section of the plume to include those spikes in the graph or analysis.

account the speed of a crab prior to receiving a concentration spike. For this reason, crabs that reach above-average velocity within 0.2 s (the shortest sampling interval) may already have been at above-average velocity prior to a spike and did not necessarily experience acceleration in response to the concentration spike.

The analysis reveals a strong link between stimulus arrival and velocity; crabs overall took less than a second (0.4 – 0.7 s) to reach above-average velocity following a spike at the antennules. Crabs in the Continuous plume took the shortest amount of time to respond to concentration spikes over the entire path, and reached above-average velocity in under 0.5 s. Crabs in the Meandering and Pulsed plumes appeared to take longer to reach above-average velocity on average (~0.6 s) but this difference across plume types is not statistically significant ($F_{2,33} = 1.63$, $p = 0.21$). Reaction time was significantly affected by plume section itself ($F_{1,21} = 11.35$, $p = 0.003$). The interactive effect of plume type and plume section on reaction time was marginally signficant ($F_{2,21} = 3.33$, $p = 0.06$). Crabs in the Continous and Meandering plumes respond two- to three-times faster in the middle section of the plume (~0.3 s) than they did in the downstream section of the plume (~0.5 and 0.7 s, respectively). Crabs in the Pulsed plume did not change reaction time regardless of plume section.

An analysis of the percent of times that crabs reached above-average velocity within one second following an antennule spike (Figure 5.5) indicated that, across all plume types, crabs reached above-average velocity within one second at least 68 % of the time. Crabs in the Meandering plume were the most likely (~86 %) to reach above-average velocity in the first second following a spike at their antennules. For crabs in the Continuous and Pulsed plumes, roughly 76% and 68% of spike events, respectively, were

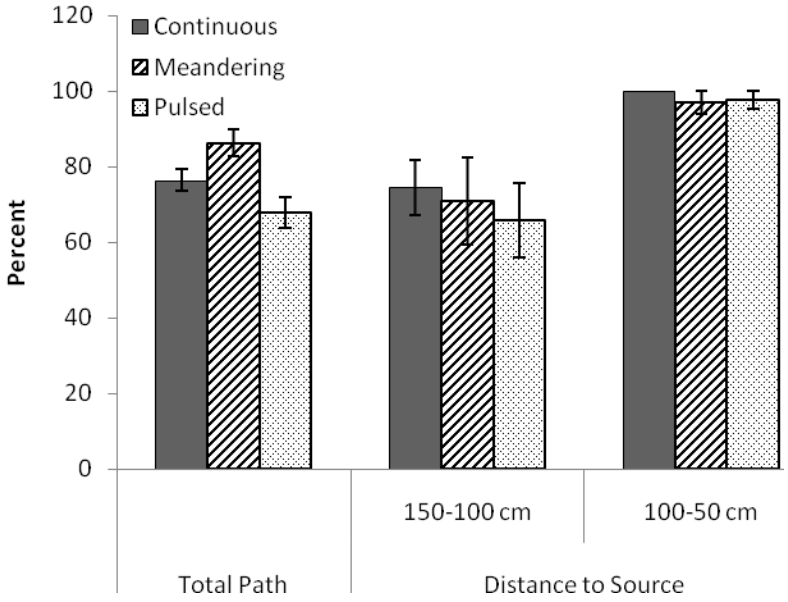

Figure 5.5. Mean percent of times crabs reached above-average velocity within one second following an antennule spike ± standard error of the mean (SEM) for crabs in various plume types. Note that there were not enough spikes in the 50-0cm section of the plume to include those spikes in the plume section analyses.

followed within one second by the crab reaching above-average velocity. This effect of plume type was significant over the total path data ($F_{2,37} = 6.63$, $p = 0.004$), which is in contrast to the previous analysis on mean reaction time (Figure 5.4). Crabs have a ~66-75 % probability of reaching above-average velocity in the downstream section of the plume regardless of plume type, and nearly 100%of animals in the middle of the plume reach above average velocity within one second following a spike at the antennules. As in the previous analysis, this effect of section is significant when the data are analyzed in a repeat measures design ($F_{1,31} = 19.90$, $p < 0.001$), but there was no significant interactive effect of plume type and plume section ($F_{2,31} = 0.005$, $p = 0.99$).

A chi-square analysis of the time it takes for a crab to reach above-average velocity following an antennule spike indicates that the time distribution is significantly affected by plume type ($\chi^2 = 22.82$, $df = 6$, $p < 0.001$) (Figure 5.6). A large frequency of

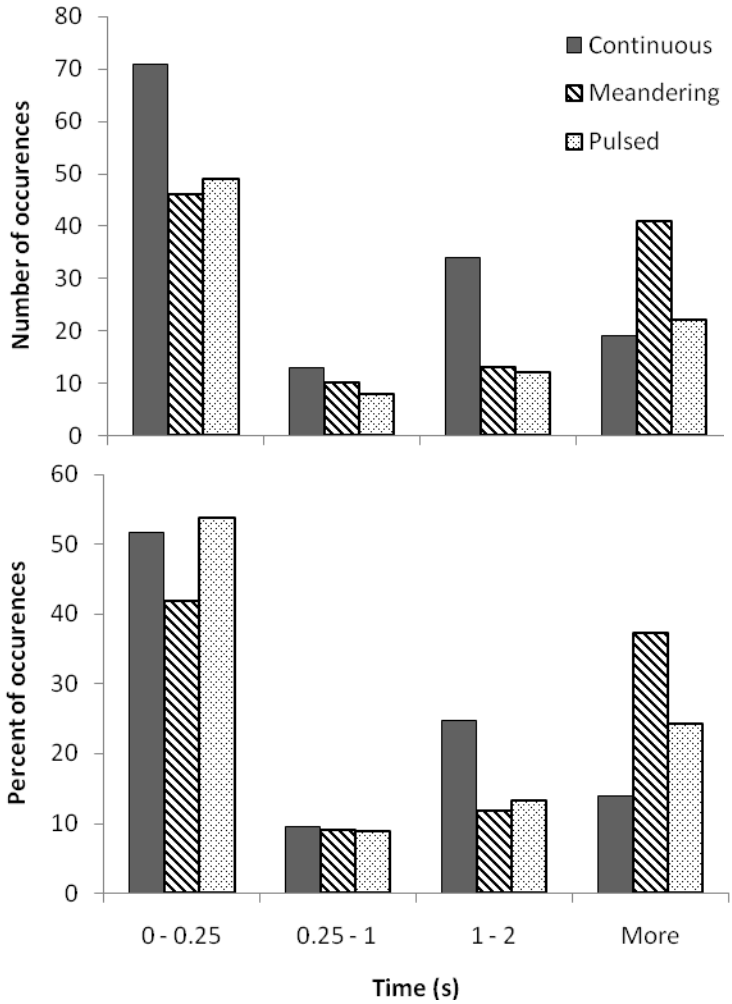

Figure 5.6. (a) Frequency and (b) percent distribution of time it takes for a crab to reach above-average velocity after receiving a spike at its antennules for crabs in various plume types. Note that, because our behavioral sampling is ~5 Hz, animals remaining at above average velocity following a spike are recorded as reaching average velocity in 0-0.25 s, as are animals that accelerated to above average velocity within 0.25 s of a spike. Subsequent time bins reflect animals that are moving at below average velocity within 0-0.25 s of receiving a spike, then accelerate to above average velocity.

crabs in all plume types reach, or are at, above-average velocity within 0.25 s after a spike and relatively few crabs in the Continuous and Pulsed plumes (25% or less) fail to reach above-average velocity within 2 s after an antennule pulse. In contrast, crab behavior in the Meandering plume displays a strong bimodal pattern with a roughly equal number of crabs responding immediately (< 0.25 s) to a concentration spike at the

antennules as the number of crabs taking a comparatively long time (> 2 s) to reach above-average velocity.

An anlysis of crabs that do not reach above-average velocity within two seconds of receiving a spike at the antennules (*i.e.*, reponse times > 2 s from Figure 5.6) was performed to determine whether these crabs were in the process of stopping or decelerating. The percent distribution of the time it takes for these crabs to either stop or reverse direction indicates that the vast majority either stopped or reversed their direction (*i.e.*, headed downstream) within one second of receiving that antennule spike (Figure 5.7). A chi-square anlysis confirms that the distribution is significantly affected by plume type ($\chi^2 = 24.8$, df = 4, $p < 0.0001$).

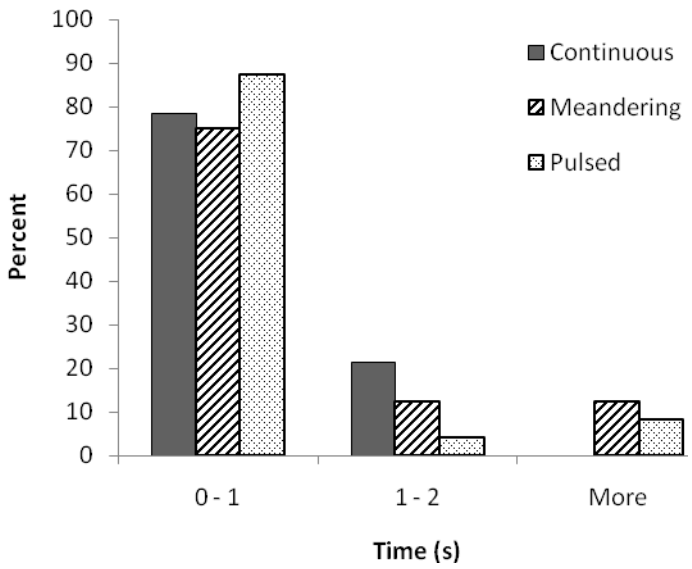

Figure 5.7. Percent distribution of the time it takes for a crab that took longer than two seconds to reach above-average velocity (nonresponsive) to stop or reverse direction (x-velocity ≤ 0.5 cm s^{-1}) follwing a spike at the antennules for crabs in various plume types.

5.4.2 Average velocity before and after receiving and antennule spike

The analysis of crabs that do not reach above-average velocity within two seconds of a spike arriving at their antennules suggests that the state of the crab as it recieves the concentration spike affects the reaction of the crab to the spike. To examime this effect, response times of crabs that were moving at either above-average velocity prior to receiving an antennule spike (Figure 5.8) or moving at below-average velocity prior to the antennule spike (Figure 5.9) were analyzed.

Crabs that were traveling at above-average velocity immediately prior to receiving a spike at the antennules (Figure 5.8) largely continued to travel at above-average velocity immediately following an antennule spike. Crabs that were traveling at below average velocity before receiving an antennule concentration spike (Figure 5.9) were roughly equally as likely to reach above-average velocity within any of the four time bins. A log-linear analysis (VassarStats) demonstrates that the full model is significant ($G^2 = 80.92$, $df = 17$, $p < 0.0001$) but that plume type does not have a significant effect on the frequency distribution of time to reach above-average velocity ($G^2 = 11.26$, $df = 6$, $p = 0.08$). As suspected, the initial conditions of the crab (*i.e.*, speed prior to receiving an antennule spike) had a significant effect on the distribution of time to reach above-average velocity ($G^2 = 65.58$, $df = 3$, $p < 0.0001$), with crabs that were previously traveling at below average velocity taking longer to reach above-average velocity.

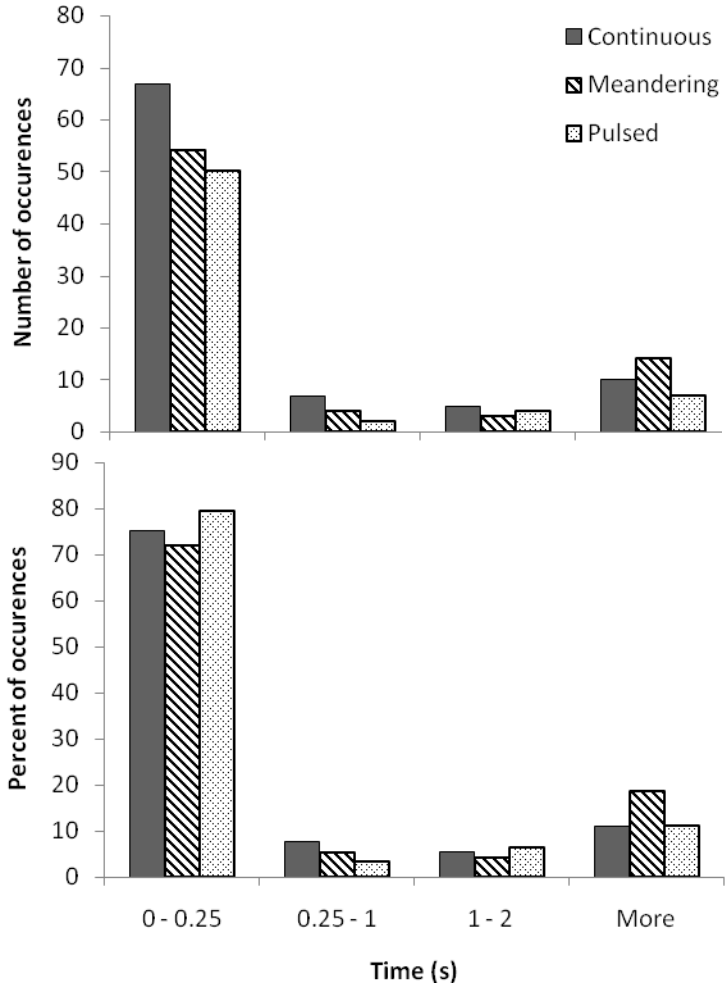

Figure 5.8. (a) Frequency and (b) percent distribution of post spike velocity patterns for crabs in different plumes. The y-axis values represent the frequency of animals that reach above-average velocity in the respective time interval after receiving a spike at their antennules for animals traveling at above-average velocity prior to receiving a spike at their antennules. Note that, because our behavioral sampling is ~5 Hz, animals remaining at above average velocity following a spike are recorded as reaching average velocity in 0-0.25 s, as are animals that accelerated to above average velocity within 0.25 s of a spike. Subsequent time bins reflect animals that are moving at below average velocity within 0-0.25 s of receiving a spike, then accelerate to above average velocity.

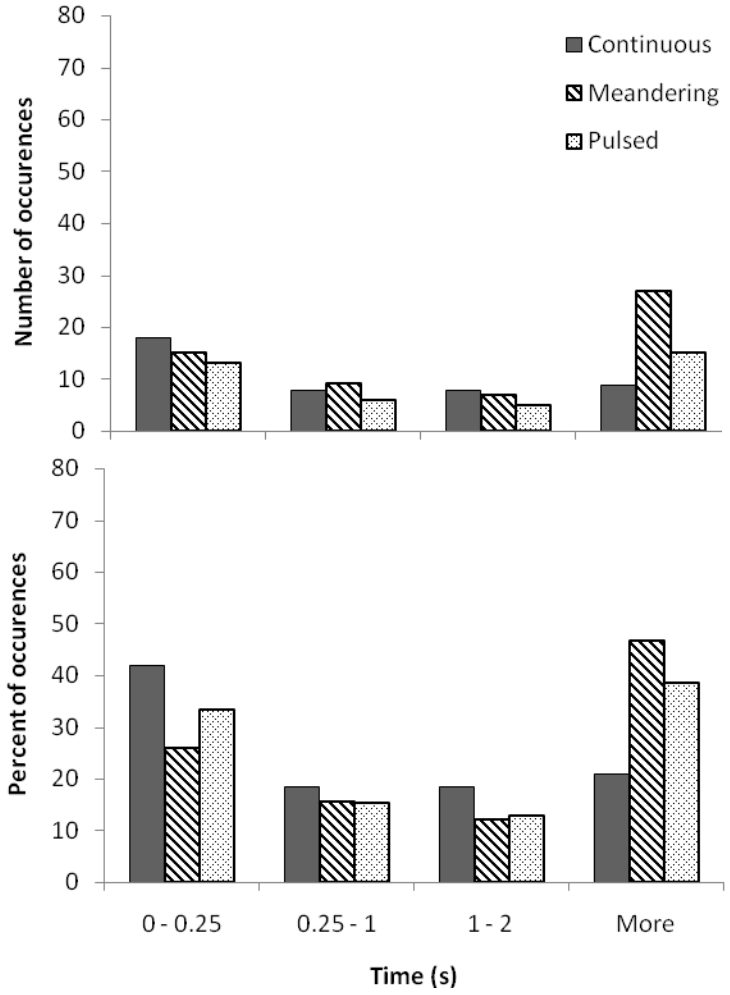

Figure 5.9. (a) Frequency and (b) percent distribution of post spike velocity patterns for crabs in different plumes. The y-axis values represent the frequency of animals that reach above-average velocity in the respective time interval after receiving a spike at their antennules for animals traveling at below average velocity prior to receiving a spike at their antennules.

5.5 Effect of prior behavior on acceleration in response to single spikes

Analyzing crab behavior in relation to the time to above-average velocity provides a good measure of strong reactions to stimulus pulses; however, this analysis may mask instances where positive responses to stimuli go unrecognized because they are more subtle. Crabs that begin to move towards the source in response to stimuli may not be recognized as positive responses if they do not reach the average velocity

threshold within a certain time period. Similarly, negative responses to stimuli (decreasing velocity) may be masked for crabs that are already well above-average velocity prior to receiving the stimuli. To eliminate these potential confounding effects, analyses were performed on the acceleration/deceleration patterns of crabs prior to and after receiving a spike at the antennules.

Frequency distributions of the post-spike acceleration patterns were analyzed for crabs that were both accelerating and decelerating prior to the antennule spike (Frequency: Figure 5.10; Percent: Figure 5.11). For animals accelerating prior to spike arrival (Figs. 5.10a and 5.11a), nearly 70% continued to accelerate in the interval 0-0.25s, whereas the remaining 30% initially decelerated and resumed acceleration at a later interval. Animals that initially were decelerating prior to a spike (Figs. 5.10b and 5.11b) were also capable of rapid acceleration; 30% accelerated within 0-0.25s after receiving a spike. This observation is consistent with the rapid response time of animals seen in earlier analysis. Animals in the Continuous plumes show the highest frequency of rapid accelerations, whereas the least frequent incidence of rapid accelerations occurs in the Pulsed plumes. A log-linear analysis (VassarStats) indicates that the full model is significant ($G^2 = 104.14$, $df = 17$, $p < 0.0001$). In addition, plume type significantly affects the frequency distribution of acceleration times following an antennule spike ($G^2 = 18.9$, $df = 6$, $p = 0.004$). However, the post spike distribution of time to acceleration is also significantly affected by pre-spike acceleration or deceleration state ($G^2 = 77.06$, $df = 3$, $p < 0.0001$); animals that are decelerating prior to a spike take longer to accelerate than animals that were previously accelerating (Figure 5.11), as evinced by the smaller proportion of rapid acceleration (0 - 0.25 s) and larger proportion of acceleration over

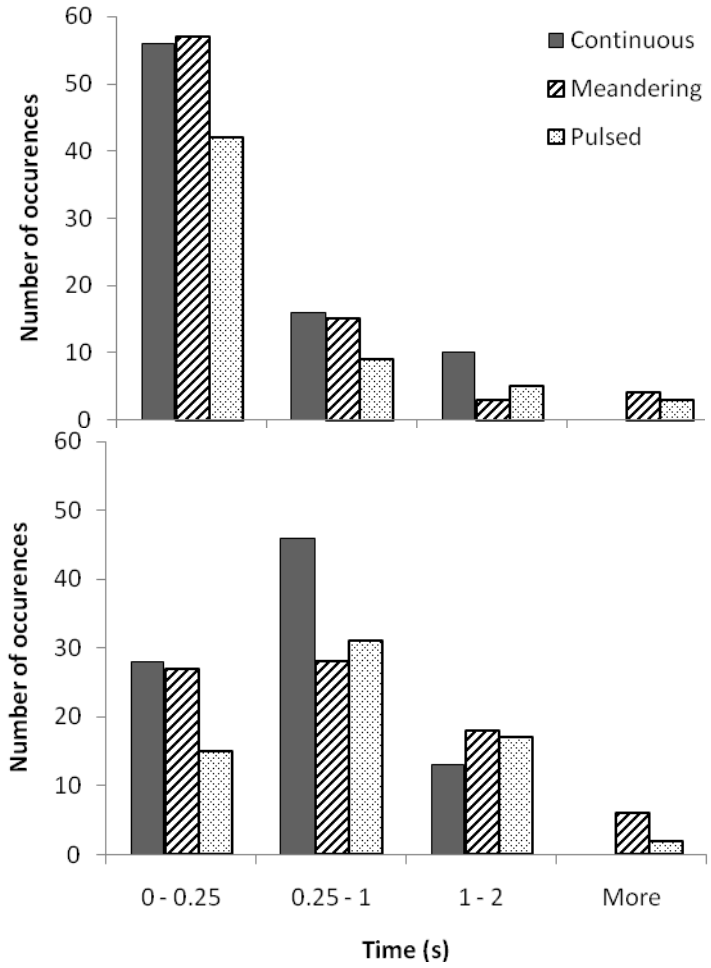

Figure 5.10. Frequency distribution of post spike acceleration patterns of crabs in different plumes. The y-axis gives the frequency of animals that accelerate within a given time interval for animals that were (a) accelerating or (b) decelerating prior to receiving a spike at the antennules in various plume types.

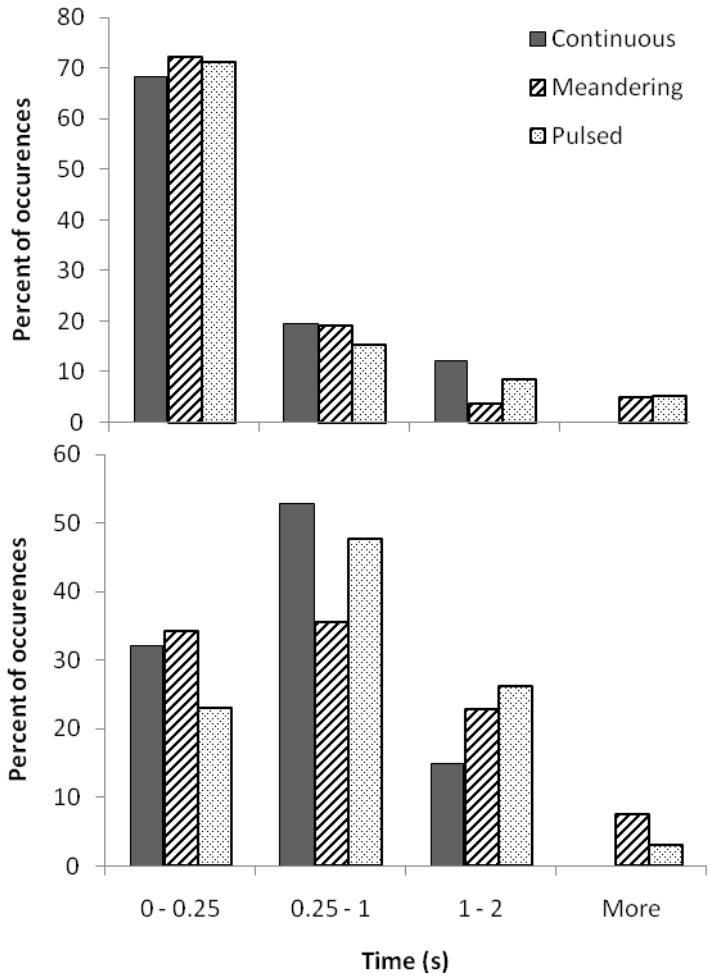

Figure 5.11. Percent distribution of post spike acceleration patterns of crabs in different plumes. The y-axis gives the frequency of animals that accelerate within a given time interval for animals that were (a) accelerating or (b) decelerating prior to receiving a spike at the antennules in various plume types.

long time courses (0.25 – 2 s). There is no interactive effect between pre-spike acceleration or deceleration and plume type ($G^2 = 0.16$, $df = 2$, $p = 0.92$).

A previous analysis (Figure 5.7) suggested that animals that do not reach above-average velocity following antennule spikes decelerate and stop. Therefore, I re-analyzed stopping/reversal times of crabs that do not accelerate within one second after receiving an antennule spike. As in the previous analysis, I incorporated both the effect of plume

type as well as the prior state of the crab, that is, whether it was accelerating or decelerating prior to receiving the antennule spike (Figure 5.12).

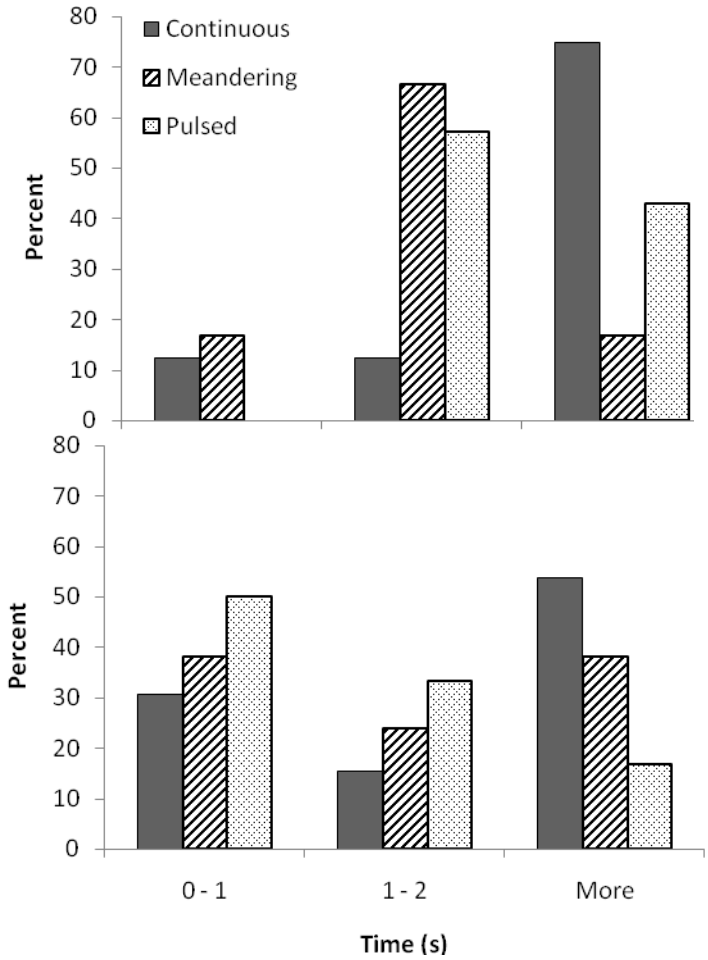

Figure 5.12. Frequency distribution of post spike stopping and reversal (x-velocity ≤ 0.5 cm s^{-1}) for crabs that took longer than one second to accelerate follwing a spike at the antennules for crabs in various plume types. The Y axis represents the frequency of animals for crabs that were (a) accelerating or (b) decelerating prior to receiving an antennule spike.

Prior behavior (acceleration or deceleration) had a significant effect on subsequent behavior ($G^2 = 7.74$, df = 2, $p = 0.02$), with animals displaying generally shorter reaction times if they were previously decelerating. Plume type did have a marginally significant effect on the frequency distribution of the time it took these crabs

to stop or reverse direction ($G^2 = 7.9$, $df = 4$, $p = 0.10$), and there was no significant interactive effect between plume type and prior acceleration or deceleration ($G^2 = 1.44$, $df = 2$, $p = 0.49$). However, the analysis of these effects (or lack thereof) is complicated by the significant effect of the whole model ($G^2 = 22.04$, $df = 12$, $p = 0.04$). Crabs in the Continuous plume were most likely to take longer than 2 s to stop or reverse direction, regardless of pre-spike acceleration or deceleration. Crabs in the Meandering plume stop or reverse with equal likelihood within 1 s and greater than 2 s regardless of whether they were accelerating vs. decelerating. Crabs decelerating in the Pulsed plume have are least likely to take longer than 2 s to stop/reverse direction, whereas crabs in the Continuous and Meandering plumes show bimodal-like patterns, with the lowest frequency of reversals at the intermediate time step (1-2 s)

5.6 Acceleration in response to spike frequency

The frequency of concentration spikes may mediate the upstream speed of crabs towards the source, with crabs responding to more frequent spikes by quickly increasing upstream velocity. Previous experiments (Chapter 2) demonstrate that crabs move towards an odor source more slowly when tracking in increasingly homogenized, less filamentous plumes, supporting this hypothesis. The same pattern shows up repeatedly in Chapter 4, including the longer search times of crabs in the Meandering and Pulsed plumes (Figure 4.4), longer stop times (Figure 4.7), and slower along-stream progression towards the source (Figure 4.13). Additionally, analysis in this chapter suggests accelerations are not always preceded by spikes (Figure 5.2). Consequently, spike and acceleration data were analyzed to determine how recently a crab received a prior antennule spike and the relationship of this interval to post spike acceleration or

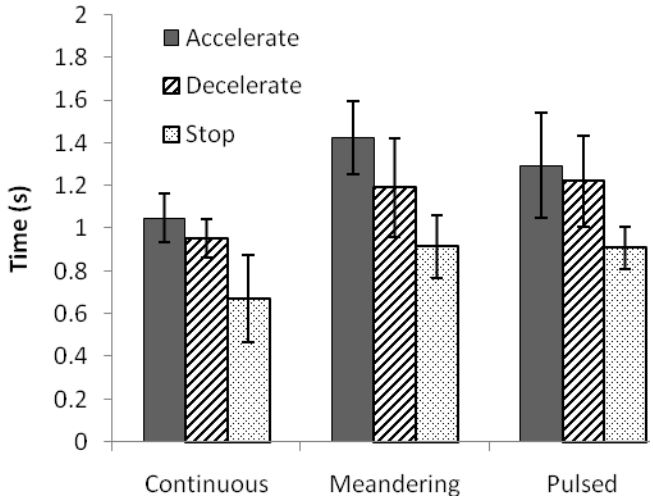

Figure 5.13. Mean interspike interval prior to receving an antennule spike ± standard error of the mean (SEM) for crabs accelerating, decelerating, or stopping in various plume types. (a) Raw data and (b) data normalized by the mean time between spikes for each plume are presented.

deceleration (Figure 5.13). Data were only analyzed for crabs moving forward at the time that they received an antennule spike. Post spike deceleration was separated by whether the crab decelerated but continued to move forward or it decelerated to a stop.

The analysis of interspike interval showed general trends indicating smaller interspike intervals actually cause animals to stop, but it did not show a significant relationship of post spike acceleration on the raw time to last spike ($F_{2,278} = 1.74$, $p = 0.18$). Similarly, there was not a significant effect of plume type on reaction time ($F_{2,278} = 0.95$, $p = 0.39$; Figure 5.13), although interspike intervals generally were longer for crabs in the Meandering and Pulsed plumes for all behaviors. Finally, there is no significant interaction effect between plume type and post spike acceleration on the raw time to last spike ($F_{4,278} = 0.07$, $p = 0.99$). Data were subsequently normalized by the mean time between spikes for each plume type, which was presented at the beginning of this Chapter (Figure 5.1). The trends remained the same, but normalizing the data did not produce significant associations between plume type, post spike response, or their

interaction and the time to last spike (Plume type: $F_{2,278} = 0.33$, $p = 0.72$; Post spike response: $F_{2,278} = 1.79$, $p = 0.17$; Interaction: $F_{4,278} = 0.06$, $p = 0.99$).

The trends revealed in Figure 5.13 suggest that significant relationships between interspike interval, plume type, and behavioral output may be difficult to detect due to the variance of interspike intervals. Thus, I performed a frequency analysis of behavioral response as a function of time to last spike (Figure 5.14). The analysis indicates that small interspike intervals frequently are associated with decelerating and stopping.

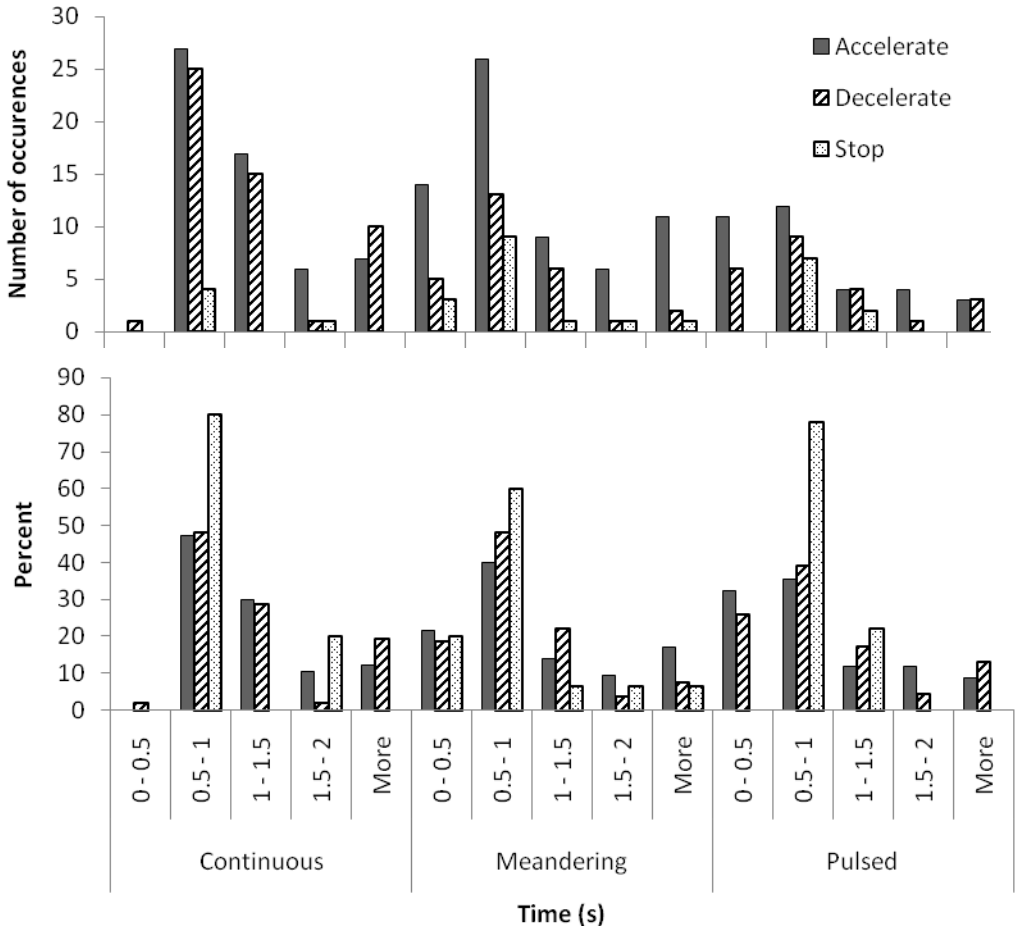

Figure 5.14. (a) Frequency and (b) percent distribution of length of time to last spike for crabs accelerating, decelerating, or stopping in various plume types.

Most interspike intervals in the Continuous plume are > 0.5 s and crabs that do stop are experiencing the shortest common interspike intervals (1 s) 80% of the time. Similarly, most instances of velocity changes in the Meandering plume are in the 0.5-1 s time interval (although there are appreciable numbers of events in the smallest increment, 0 – 0.5 s), regardless of what that velocity change may be. Even though the absolute frequency of certain interspike intervals is different, the percent distribution reveals a very similar (indiscriminate) relationship between interspike intervals and all types of velocity changes, indicating that raw interspike interval does not predict the forward velocity of crabs in the Meandering plume. Nonetheless, the smaller interspike intervals are again associated with accelerating or decelerating with roughly equal frequency, and the majority of stops happen at intervals of 1 s. The percent distribution of the interspike intervals across velocity changes looks similar in the Pulsed plume as well. Again, interspike intervals of less than 0.5 s are associated with crabs accelerating and decelerating whereas the majority of stopping events occur at intervals of 1 s.

The percent distribution of the interspike intervals associated with various velocity changes looks similar in Continuous and Pulsed plumes, but acceleration or deceleration in the Pulsed plume is associated with interspike intervals of less than 0.5 s (these events are rare in the Continuous plume). Plume type has a marginally significant effect on the interspike interval distribution ($G^2 = 14.56$, $df = 8$, $p = 0.07$). There is a significant association between post spike response and the time to last spike ($G^2 = 17.88$, $df = 4$, $p = 0.001$) and a significant interaction effect between plume type and post spike response ($G^2 = 30.76$, $df = 8$, $p = 0.0002$). In summary, the available evidence suggests that long interspike intervals are not associated with deceleration or stopping. Short

intervals produce the highest frequency of these behavioral changes, but may also be associated with accelerations, suggesting the importance of other factors in modulating the response to spike frequency.

5.7 Acceleration relative to spike concentration

As mentioned previously in the chapter on tracking behavior in bed roughness generated turbulence (Chapter 2), the concentration of individual spikes appears to have less of a governing effect on forward motion than does the frequency of those spikes. I analyzed the effects of several measures of change in spike concentration on movement to determine whether spike concentration was used by animals as a movement cue. The change in concentration between the current antennule spike and the most recent concentration spike is a measure of whether signals are getting progressively stronger or weaker (ΔC_{Amax}, from; Figure 5.15, where $t_{0\ and}\ t_{-1}$ denote current spikes and the first prior

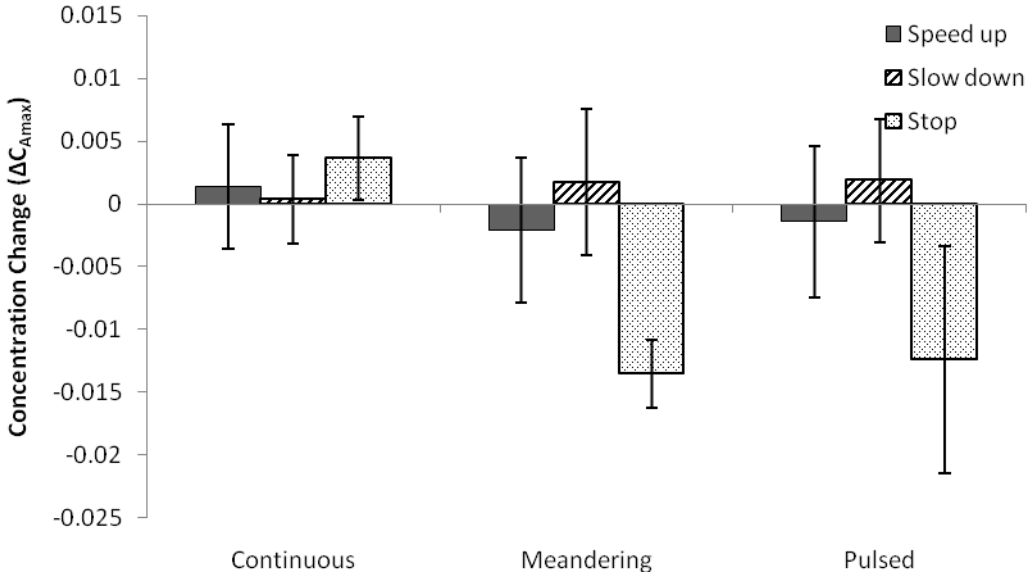

Figure 5.15. Change in concentration between prior and current antennule concentration spikes ± standard error of the mean (SEM) for crabs in various plume types.

spike respectively). There were no significant associations of plume type ($F_{2,279} = 0.66$, $p = 0.54$), post spike acceleration ($F_{2,279} = 0.68$, $p = 0.51$), or their interaction ($F_{4,279} = 0.29$, $p = 0.89$) and whether a crab was experiencing increasing or decreasing signal concentration. There is an indication that crabs in the Meandering and Pulsed plumes may be more likely to stop tracking if receiving a subsequent spike of decreased concentration (*i.e.*, crabs that stop show relatively strong negative concentration changes) but there is substantial variability in the data.

The tendency for crabs in the Meandering and Pulsed plumes to stop in response to decreasing spike concentrations is maintained when behavior is examined with respect to concentration change through time ($\Delta C_{Amax}/\Delta t$, from t_{-1} to t_0; Figure 5.16).

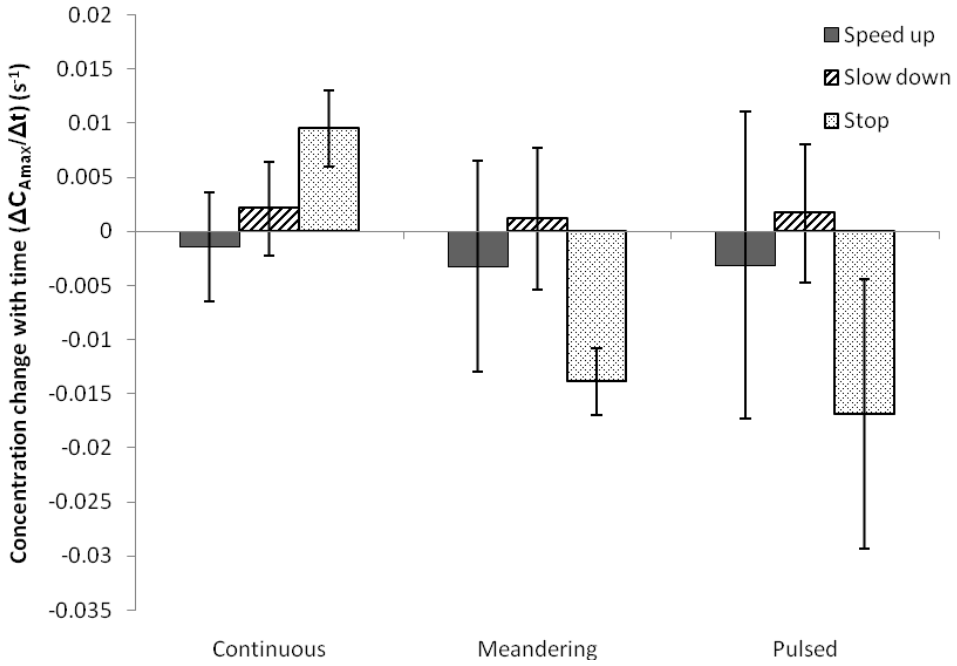

Figure 5.16. Change in concentration between prior and current antennule concentration spikes with time ± standard error of the mean (SEM) for crabs in various plume types. when they experience increasing concentrations with time.

Conversely, crabs in the Continuous plume appear to stop when they experience increasing concentrations with time. Although this trend remains, there is still no significant effect of plume type ($F_{2,279} = 0.69$, $p = 0.50$), post spike acceleration ($F_{2,279} = 0.52$, $p = 0.59$), or their interactive effect ($F_{4,279} = 0.31$, $p = 0.87$) on the level of increasing or decreasing signal concentration a crab receives with time.

A final concentration analysis examined whether tracking crabs demonstrated a predictable reaction to the change in concentration change over a period of time, in this case over the time period of two previous spikes ($\Delta(\Delta C_{Amax}/\Delta t)$,, from t_{-2} to t_{-1} and from t_{-1} to t_0; Figure 5.17). This analysis also failed to find any significant effect of plume type

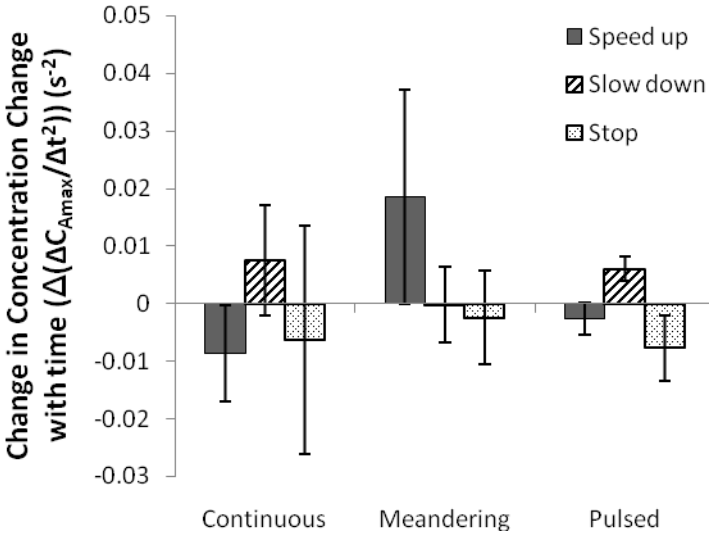

Figure 5.17. Mean change in concentration change with time between last two antennule spikes before current antennule spike ± standard error of the mean (SEM) for crabs in various plume types.

($F_{2,287} = 0.21$, $p = 0.81$) or post spike acceleration ($F_{2,287} = 0.20$, $p = 0.82$) on whether crabs had experienced a concentration change that increased or decreased with time. There was also no interactive effect between plume type and post spike acceleration ($F_{4,287} = 0.52$, $p = 0.72$).

5.8 Integrating spike encounter at antennule and leg chemosensors

Prior research has indicated that blue crabs utilize information from their antennule chemosensors to mediate upstream motion and signals from their leg chemosensors to modulate cross-stream motion (Keller *et al.* 2003). The simultaneous collection of concentration records at both sets of chemosensors, coupled with behavioral measurements, allows us to investigate the relative contribution of antennule vs. leg chemosensors to each aspect of movement. Incorporating analysis of patterns at these different appendages jointly may also resolve some of the ambiguity seen when only responses at the antennules are examined (*e.g.*, Figs. 5.3 and 5.14).

The role of both the antennule and leg chemosensor information in the motion towards the source was investigated by analyzing the time it takes a crab reach above-average velocity following a spike at the antennules (Figure 5.4, Total Path data) as a function of spikes at its antennules or legs during the previous two seconds (Figure 5.18). This analysis employed a 3-way ANOVA with main effects consisting of plume type, the presence/absence of a prior leg spike, and the presence/absence of a prior antennule spike.

Interestingly, receiving a prior spike at the legs within one second does have a significant effect on the time it takes for a crab to reach above-average velocity following an antennule spike ($F_{1,99} = 5.63$, $p = 0.02$), though there is no significant interactive effect between plume type and previous leg spike ($F_{2,99} = 0.01$, $p = 0.99$). Receiving a previous spike at the antennules within one second has a marginally significant effect on time to above-average velocity ($F_{1,99} = 3.26$, $p = 0.07$). The response to an antennule spike for any given combination of prior leg or antennule spikes is the same regardless of plume

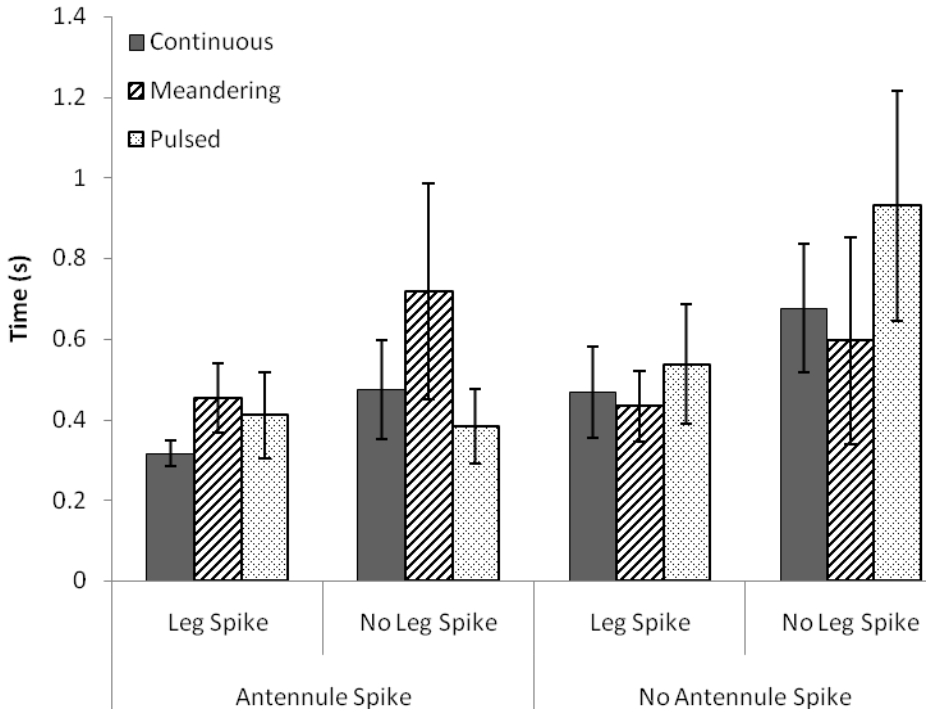

Figure 5.18. Mean time that it takes crabs to reach above-average velocity within two seconds following an antennule spike as a function of spike reception ± standard error of the mean (SEM) for crabs in various plume types. Data are analyzed as a whole and separated by whether the crab received a concentration spike at its antennules and/or legs within one second preceding the antennule spike.

type ($F_{2,99} = 0.43$, $p = 0.66$) and there is no significant effect of the interaction between plume type and previous antennule spike ($F_{2,99} = 1.90$, $p = 0.16$). There is no significant two-way interactive effect between receiving a prior antennule spike and receiving a prior leg spike ($F_{1,99} = 0.57$, $p = 0.45$) and no significant three-way interactive effect of plume type with prior reception of an antennule or leg spike ($F_{2,99} = 0.83$, $p = 0.44$). In summary, receiving a prior spike at either the legs or (probably) the antennules reduces the response time of crabs receiving a subsequent antennule spike regardless of plume type, and the response time is not further reduced if animals receive prior spikes at both legs and antennules.

5.9 Forward motion results summary

Correlating simultaneous plume and behavior data from tracking blue crabs has provided insight into a complex system of signals and responses. Blue crabs appear to rely on reception of odorant bursts at their antennule and leg sensors to mediate upstream motion, but do not modify their behavior based on the absolute concentration of those bursts. Their responses are extremely rapid and they are able to change their behavior within a half a second or less of receiving a stimulus. There is also evidence that blue crabs utilize state-dependent responses to mediate their upstream motions, differentially responding to signals based on their current behavior at the time of signal reception.

5.9.1 Upstream velocity and antennule spike reception

Initial examination of this data supports a positive relationship between upstream velocity and antennule spike reception. An analysis of interspike interval at the antennules (Figure 5.1) reveals that crabs in the Meandering and Pulsed plumes experience significantly longer times between spikes than do crabs in the Continuous plume. Crabs in the Continuous plume also spend significantly less time searching for an odor source (Figure 4.4), supporting the hypothesis that there is a positive link between upstream velocity and spike reception at the antennules. The highly increased time between spikes for crabs in the Meandering and Pulsed plumes is not surprising given the source characteristics of those plumes. As discussed in Chapter 4, the discontinuous nature of the Pulsed plume ensures that crabs will lose contact with the plume for extended periods of time, which will affect the average interspike interval. Crabs in the Meandering plume experience ambiguity of plume source direction as levels of intermittency are not an indicator of proximity to the plume centerline (Dickman 2008)

and basic path data indicate that crabs in the Meandering plume spend the longest time tracking (Figure 4.4) and stop with the greatest frequency (Figure 4.6). If contact with plume filaments at the antennules is necessary to sustain upstream movement, this indicates that the difficulty tracking the Meandering plume results in part from loss of plume contact, which increases the intervals between spikes (Figure 5.1).

5.9.2 Antennule spikes preceding upstream acceleration events

An overall positive link between spike reception and upstream velocity is challenged by the results of the analysis of spikes preceding upstream acceleration events (Figs. 5.2 and 5.3). I expected that concentration spikes at the antennules would immediately (within one second) precede acceleration events. In the Meandering and Pulsed plumes, it is much more likely that the one second preceding an acceleration event is devoid of any antennule signal (Meandering and Pulsed: ~65-70 % of the time; Continuous: ~40 % of the time). This initially seems to contradict the current hypothesis, that there is a negative link between spike reception and upstream acceleration. However, this information may be reconciled in light of the evaluation of the role of both leg and antennule sensors in upstream motion (Figure 5.18).

Reception of signals at the leg chemosensors helps to mediate upstream motion and this input is particularly important when crabs do not receive spikes at their antennules (Figure 5.18). The apparent "response" of crabs to accelerate upstream in the absence of antennule signals may actually be modulated by signal reception at the upstream legs, stimulating them to move forward. This interpretation is supported by the analyses of spike encounter related to average velocity suggesting crabs velocity is indeed related to antennule spike reception (Figs. 5.4 and 5.5). On average, crabs that do

receive spikes at their antennules are at above-average velocity within one second following the spike (Figure 5.4) and on the order of 75 % of crabs across all plumes are at above-average velocity within one second following an antennule spike (Figure 5.5). A state-dependent response to antennule signals is indicated by the acceleration data: crabs that were previously accelerating before receiving an antennule spike continue to accelerate after receiving an antennule spike (Figure 5.10) while crabs that were previously decelerating accelerate within 2 s (Figure 5.11). Although it is currently not possible to fully explain the origin of this state-dependency, it clearly can result in some decoupling between spike reception and subsequent acceleration in tracking animals.

5.9.3 Stopping and backwards motion following single antennule spikes

The data indicate that any current spike information from the antennules that would normally stimulate forward motion also may cause these crabs to cease forward motion, suggesting either state-dependent responses due to prior stimulus history, or other chemical signal inputs that suppress forward movement when combined with antenullary stimulation.

An overwhelming percent (75 % or more) of crabs that fail to reach above-average velocity within two seconds following an antennule spike (Figure 5.6, response times > 2 s) do so because they stop or reverse direction in response to the antennule spike (Figure 5.7). This latter group is in fact responding with rapid deceleration in response to an antennule spike, and the dichotomy between animals that speed up versus slow down rapidly is based on the state of the crab prior to receiving the spike. Crabs that are not already at above-average velocity prior to receiving a spike are those that respond to stimulation by stopping. This suggests that looking at crab velocity using this

measure may not tell us the most about the crab's response to individual concentration spikes.

Patterns of acceleration reinforce the observation of state dependent responses suggested above. It appears that crabs that are accelerating largely continue to accelerate after receiving an antennule spike (Figure 5.10), while crabs that have been decelerating take longer to accelerate after receiving an antennule spike (Figure 5.11). Earlier data demonstrate that crabs are capable of responding rapidly to stimuli. In particular, crabs traveling at below average velocity are able to accelerate to above average velocity within 0.25 s of receiving an antennule concentration spike (Figure 5.9). Additionally, crabs decelerating prior to receiving an antennule spike are capable of accelerating within 0.25 s of receiving an antennule spike (Figure 5.10). These data suggest that the post spike acceleration patterns (Figs. 5.10 and 5.11) of blue crabs are an effect of signal processing and not simply a time lag due to a motor effect. Therefore, the post spike acceleration patterns contradict the simple hypothesis that receiving an antennule spike should stimulate upstream movement, since this hypothesis would imply that even crabs that are slowing down should immediately accelerate in response to a spike.

5.9.4 Slowing in response to antennule spike frequency

An analysis of spike reception frequency revealed a notable trend that, across all plume types, more frequent spike reception was associated with crabs slowing down and often stopping (Figure 5.13). Crabs in the Continuous plume are the least likely to stop, although those that do tend to experience interspike intervals between 0.5 and 1 s, which is the same interval that crabs in the Pulsed plume most likely respond to with a stop (Figure 5.14). In both cases, there is little discrepancy between the interspike time

distributions of the crabs that speed up and slow down, and crabs speed up or slow down in response to interspike intervals between 0.5 and 1 more frequently than they stop in response to these intervals. The length of time to last spike is not predictive of forward velocity for crabs in the Meandering plume type as the general distribution of velocity events (*i.e.*, speed up, slow down, stop) are very similar across all interspike times (Figure 5.14b). Overall, it appears that raw interspike intervals alone do not predict the velocity responses of crabs, providing further evidence that blue crabs utilize a state-dependent response to individual odor spikes.

5.9.5 Upstream velocity and concentration

Any attempt to connect the concentration of the spikes before a velocity event (*i.e.*, speed up, slow down, stop) to the event itself produced no significant link between the concentration and plume type or particular post spike speed event (Figs. 5.15, 5.16, and 5.17) due to the high levels of variability in filament concentration; however, in the absence of significant absolute information, examining trends may provide some general information. The change in concentration spikes between the prior and current spikes (Figure 5.15) indicates that crabs that experience decreasing concentration in the Meandering and Pulsed plumes are more likely to stop but the concentration change has no apparent effect on crabs in the Continuous plume. Decreasing filament concentration is associated with increasing distance downstream of the plume source for all plume types (Dickman 2008). Under the hypothesis that forward velocity is somehow tied to filament reception of signals, I would expect decreasing concentration to be a negative signal to crabs, causing them to slow down significantly or stop if they perceive that they are moving away from the plume source. Crabs in the Continuous plume do not

experience decreasing concentration between spikes on average. This is indicative that these crabs are able to remain in contact with the plume more consistently than crabs in the Meandering or Pulsed plumes, and that the concentration of the plume filaments in the Continuous plume follow a predictable pattern of increasing concentration with increasing proximity to the source. There is some indication that crabs in the Continuous plume actually stop in response to a more dramatic increase in filament concentration, which may be indicative of being close to the source, thereby modulating a switch to near source search behavior.

The patterns seen in the basic change in concentration data are enhanced when the data are normalized by the time between the concentration spikes (Figure 5.16). Crabs that are in the Meandering and Pulsed plumes are more likely to stop if they experience a sharp decrease in concentration with time. In contrast, crabs in the Continuous plume are more likely to stop if they experience a sharp increase in concentration with time. All such patterns are eliminated in the analysis of change in concentration change (*i.e.*, change between concentration from t_{-2} to t_{-1} versus t_{-1} to t_0; Figure 5.17). This indicates that the most recent spike events play a role in determining forward motion of blue crabs while tracking but that the crabs do not seem to be utilizing changes over a longer period.

CHAPTER 6

CROSS-STREAM MOTION RESULTS FROM SIMULTANEOUS, 3DLIF MEASUREMENTS

6.1 Introduction

Animals that track in flow using odor-gated rheotaxis integrate mechanosensory information to derive the mean flow vector, and are therefore able to move upstream in response to receiving odorant stimuli (Zimmer-Faust *et al.* 1995; Keller *et al.* 1993). The generalized up-current movement in response to odor stimulation moves animals towards the source, but animals must utilize some complementary mechanism that helps them steer relative to the plume in order for odor-gated rheotaxis to be a viable strategy. Several studies in blue crabs (*e.g.*, Keller *et al.* 2003, Jackson *et al.* 2007) propose bilateral comparison as the driving force behind this steering mechanism, whereby signals from chemosensors on one side of the body are compared to signals on the opposite side and movement is accordingly directed towards the chemosensors experiencing the highest odorant concentration. Basil and Atema (1994) examined the bilateral signals available to a tracking lobster by outfitting the lobster with dopamine sensing electrodes that sampled the signals arriving in the area directly over each lateral antennule. They concluded that lobsters were responding to the onset slopes of bilateral concentration information and generally turning in the direction of the sharpest slope. While this experiment measured the concentration onset slope generally arriving at the antennules while a lobster is tracking, it fails to provide information about the properties

of the signals arriving at all the chemosensors simultaneously, which is what is necessary to develop an complete picture of how animals integrate all the information available to them to produce successful navigation behavior. Our simultaneous, three-dimensional laser induced fluorescence system provides the best opportunity thus far to examine this specific hypothesis and the general mechanisms behind blue crab orientation in odorant plumes. The body angle of blue crabs while tracking in these plumes would often prevent the crabs themselves from making cross-stream comparisons between both sets of legs as they limit the exposure of the downstream legs. Hence, I hypothesized that blue crabs could make these gradient comparisons over a single set of legs oriented in the cross-stream direction. I tested this hypothesis by analyzing turning in response to the concentration gradient across the upstream leg chemosensors.

The three source release types enable spatial and temporal comparisons of plume structure with resulting behavior. Specifically, the Continuous plume provided basic comparisons of plume properties with resulting behavior, which could then be compared to the other plumes. The Meandering plume was used to test the effects of a wider plume with large-scale spatial intermittency, specifically creating a plume where high levels of intermittency and strong concentration was not necessarily coincident with the plume centerline. The Pulsed plume was used to test the effects of large-scale temporal intermittency on crab tracking behavior. Bed roughness experiments (Chapter 2) indicated that blue crabs increase transverse movement (lower NGDR; Fig 2.1a) in response to plume intermittency caused by increased turbulence intensity. The spatial and temporal intermittency introduced by the Meandering and Pulsed plumes, respectively, should induce turning behavior in crabs tracking these plumes and provide

excellent opportunities to determine the signals that control this turning behavior by comparison to tracking in the Continuous plume.

6.2 Heading angle

The frequency distribution of the crab's heading angle was examined for each plume type to determine if a crab's steering is differently affected as a function of chemical signal structure patterns (Figure 6.1). The heading angle (α = angle between the vector pointing from the crab to the source (R) and a vector pointing in the direction of motion (r); *see* Chapter 3, Section 3.1, for a more detailed explanation of heading angle) is a measure of how directly a crab is moving towards the source. Positive heading angles indicate crabs are moving towards the plume centerline and negative heading angles indicate the crab is moving away from the plume centerline, with 0˚ representing the crab heading directly towards the source and ± 180˚ representing the crab heading directly away from the source. All plume types display a strong peak in heading angles at around 0º (crab moving directly towards the source), with the majority of turns occurring at small heading angles (between -45 and 45˚). There is a bias towards positive heading angle corrections, indicating that the crabs preferentially make minor course corrections towards the plume centerline. Both the Meandering and Pulsed plume heading angle distributions show smaller local peaks around -90 and 90˚ (crab is moving sideways and making no net movement towards the source or away from the source), whereas the distribution for crabs in the Continuous plume shows only a small local peak around 90˚. Downstream movements (*i.e.,* turns < 90 or > 90˚) are extremely rare in all plume types but essentially nonexistent in the Continuous plume. There is an increase in

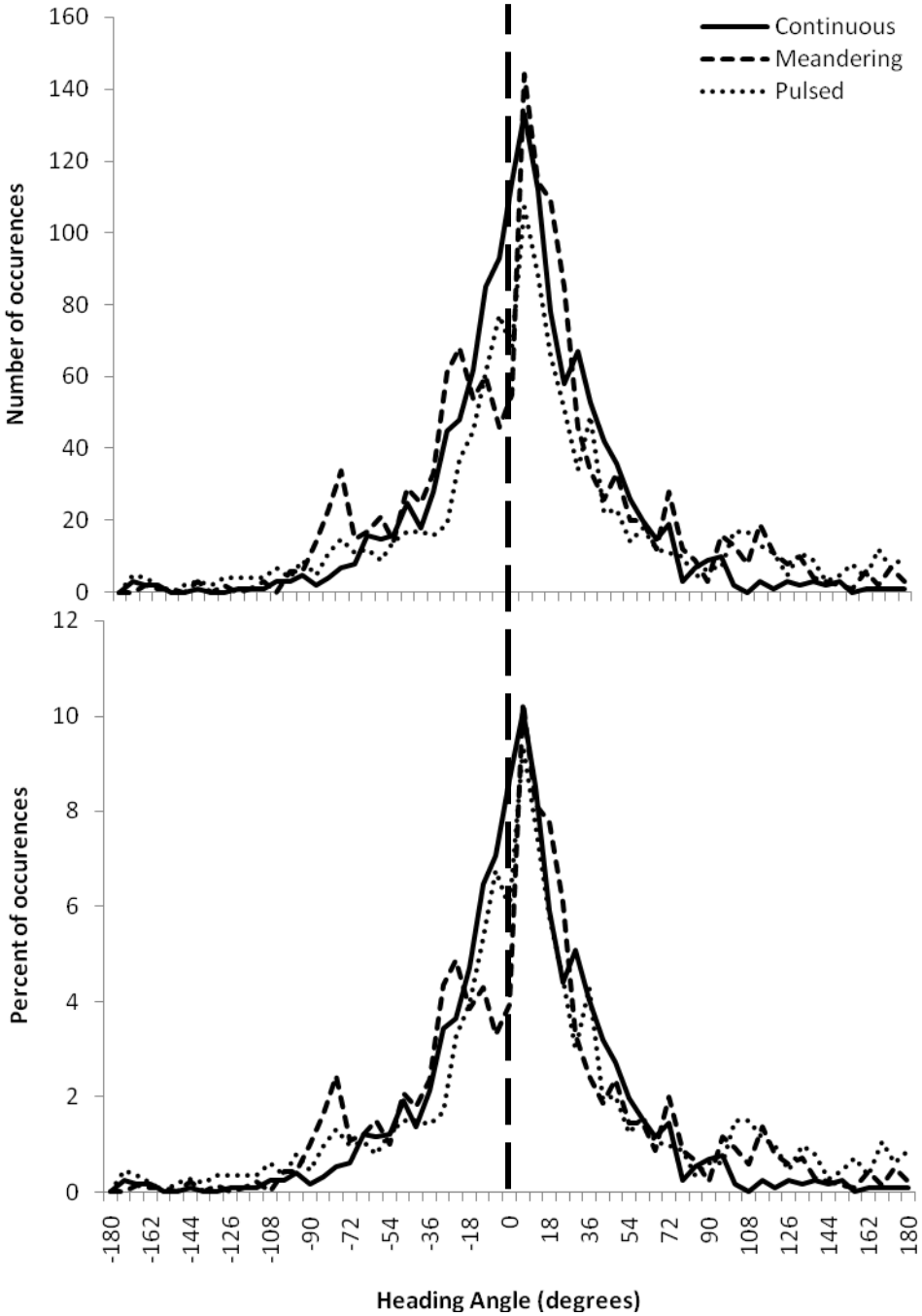

Figure 6.1. (a) Frequency and (b) percent distribution of heading angle (α) for the total length of the plume for crabs in various plume types. The vertical dashed line represents the 0˚ mark. Heading angles to the left of this mark (-) indicate that the crab is headed away from the plume centerline and heading angles to the right of this mark (+) indicate that the crab is headed towards the plume centerline.

positive angles (course corrections towards the center of the plume) higher than 90˚ in both the Meandering and Pulsed plumes.

Crabs in the Meandering and Pulsed plumes have a very strong peak at small positive angles and a much more minor peak at small negative angles. In the Meandering plume, the peak at small, negative heading angles (*i.e.*, $-45^\circ < \alpha < 0^\circ$) is roughly half the magnitude of the peak at small, positive heading angles (*i.e.*, $0^\circ < \alpha < 45^\circ$). In the Pulsed plume, the peak at small negative heading angles is only about two-thirds the magnitude of the small positive heading angle peak. These small negative peaks (absent in the Continuous plume) indicate that crabs in the Meandering and Pulsed plumes have a tendency to make small path corrections away from the plume source, suggesting that these crabs have greater confusion about the plume source direction. The absolute degree at which crabs in the Meandering plume make small negative course corrections is greater than the absolute value at which they make small positive course corrections. This indicates that crabs in the Meandering plume make more extreme course corrections away from the source (negative) than towards the source (positive), especially compared to the crabs making small course corrections in the Continuous and Pulsed plumes.

It is important to examine the heading angle of crabs at various points as they approach the source as the along-stream proximity of the crab to the source has the potential to change the heading angles of crabs, both due to the geometry itself and the active width of the plume. In the downstream (150-100 cm downstream of the source) section of the plume there is still a tendency for the heading angle distributions to skew towards positive (towards the plume centerline) angles (Figure 6.2). The Continuous distribution peaks at nearly 0˚, with a high frequency of angles at small, positive headings (between 6 and 12˚), with a strong secondary peak 24 and 36˚. The local peak at small negative angles in the Meandering plume distribution is appreciable relative to the major

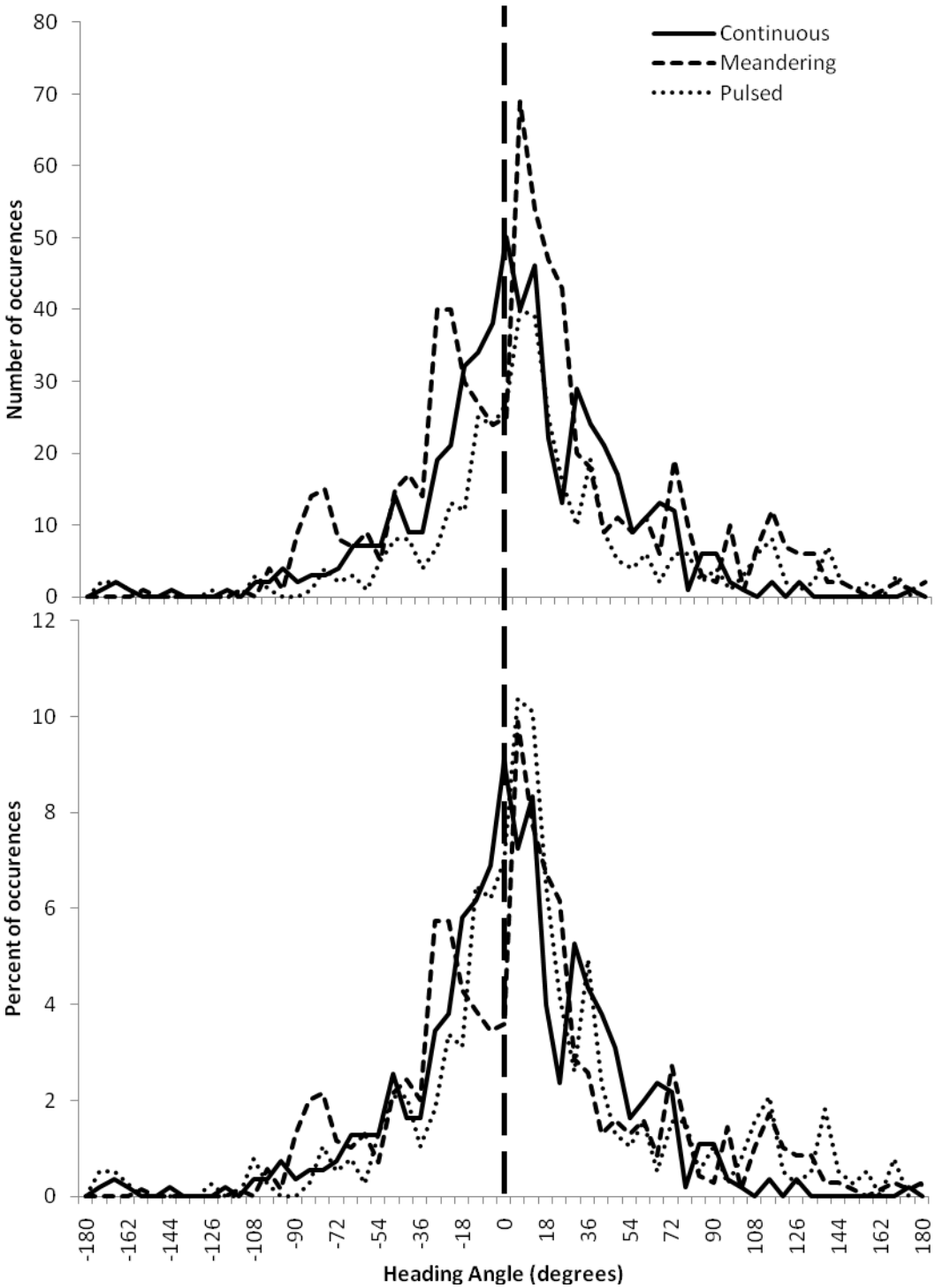

Figure 6.2. (a) Frequency and (b) percent distribution of heading angle (α) for the downsteam section of the plume (150-100 cm from source) for crabs in various plume types. The vertical dashed line represents the 0˚ mark. Heading angles to the left of this mark (-) indicate that the crab is headed away from the plume centerline and heading angles to the right of this mark (+) indicate that the crab is headed towards the plume centerline.

peak at small positive angles. This indicates that crabs in this section may have difficulty determining the direction of the plume centerline and are more likely to make strong negative course corrections than crabs in the Continuous or Pulsed plume. Continuous and Pulsed distributions retain the distribution that they displayed in the frequency distributions of heading angle from the total path data (Figure 6.1), characterized by a strong peak near 0˚, and a bias towards positive angles. The local peak around -90˚ in the Pulsed plume has been damped and thus no longer a prominent feature, though there are still local peaks in the Pulsed plume around 90˚. Angles indicating movement downstream (less than -90 or greater than 90˚) are almost exclusively positive in all plumes, indicating that crabs are correcting towards the plume centerline when they do move downstream.

Heading angle distributions for the three plume types appear to converge (Figure 6.3) in the middle section of the plume (100-50 cm downstream from the source). There again is a skew towards positive heading angles and the distributions peak at small, positive angles. All plumes show a local peak in heading angle frequency at 90˚. The distributions of heading angles in Meandering and Pulsed plumes again show local peaks at ± 90º that are not present in the Continuous plume distribution. There is still a strong tendency for crabs that move downstream to tend towards positive angles (towards the plume centerline) though crabs in the Pulsed plumes are more likely to move downstream at negative heading angles than the crabs in the other plumes.

The heading angle frequency distributions are very different among the three plume types (Figure 6.4) in the upstream section of the plume (50-0 cm from the source). In the Continuous plume there is a strong and sharp peak at small, positive heading

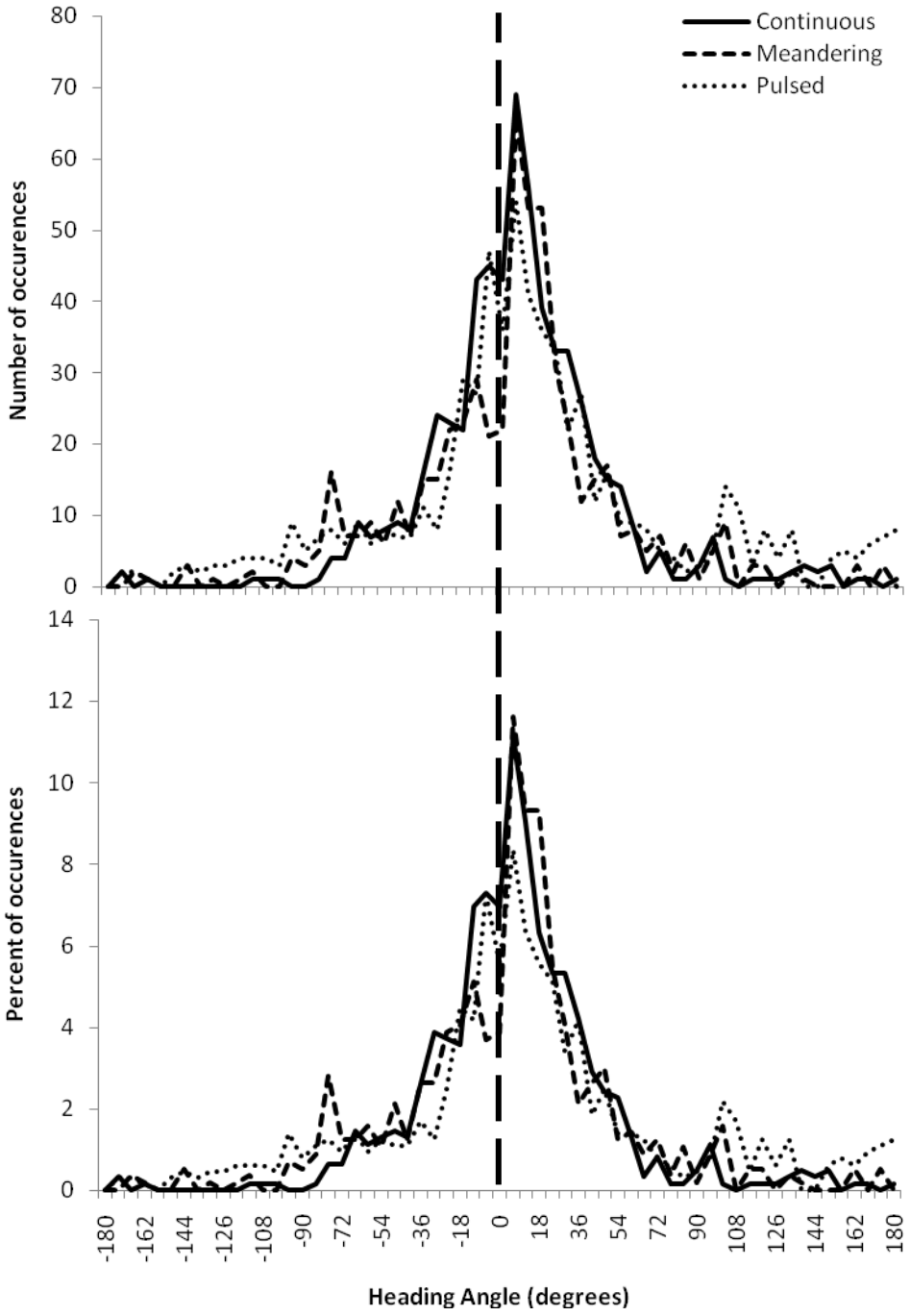

Figure 6.3. (a) Frequency and (b) percent distribution of heading angle (α) for the middle section of the plume (100-50 cm from the source) for crabs in various plume types. The vertical dashed line represents the 0˚ mark. Heading angles to the left of this mark (-) indicate that the crab is headed away from the plume centerline and heading angles to the right of this mark (+) indicate that the crab is headed towards the plume centerline.

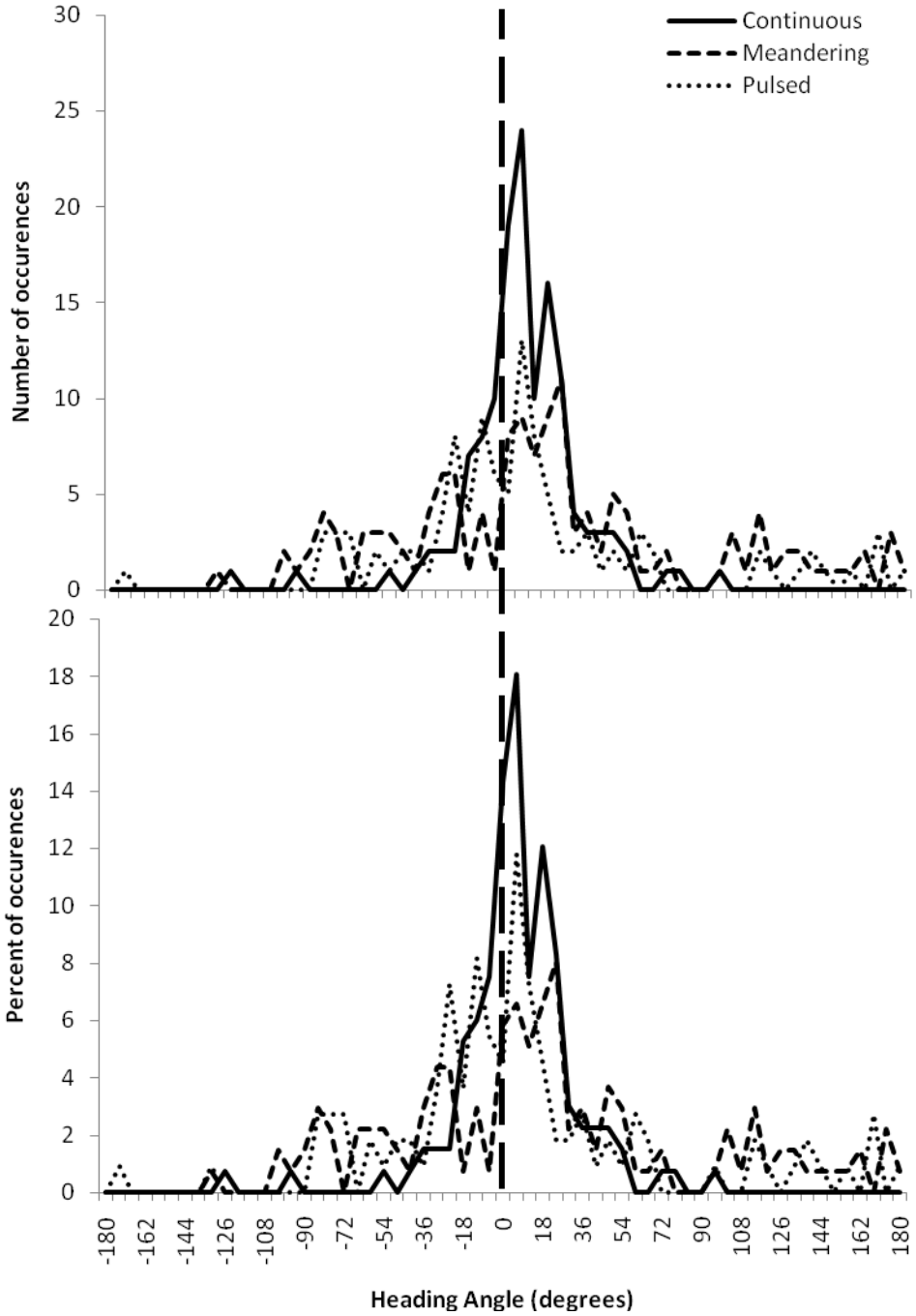

Figure 6.4. (a) Frequency and (b) percent distribution of heading angle (α) for the upstream section of the plume (50-0 cm from the source) for crabs in various plume types. The vertical dashed line represents the 0˚ mark. Heading angles to the left of this mark (-) indicate that the crab is headed away from the plume centerline and heading angles to the right of this mark (+) indicate that the crab is headed towards the plume centerline.

angles and a general skew towards positive angles overall. This indicates that crabs in the Continuous plume make accurate course corrections towards the plume centerline when they are very close to the source. A bimodal trend again has emerged, where there is a second local peak in smaller, positive heading angles (between 12 to 18˚). There are no local peaks around -90 or 90˚ indicating little direct sideward motion, and virtually no instances where the crab is moving downstream either towards or away from the source (*i.e.*, at positive or negative heading angles > 90). The Meandering and Pulsed plumes show distributions with many local peaks and no clear tendency towards positive or negative angles overall. However, both distributions indicate that crabs in these plumes that move backwards do so at positive heading angles. The maxima of the Meandering and Pulsed distributions still fall within small, positive angles indicating turns upstream towards the source.

The preceding analysis suggests the three different plume types evoke substantially different tracking behaviors. Crabs in the Continuous plume make increasingly small, positive course corrections as they move upstream, indicating that their sense of the location of the plume centerline improves as they approach the source. Crabs in the Meandering and Pulsed plumes are the most likely to make course corrections that directed away from the source. Contrary to the tracking pattern of crabs in the Continuous plume, the distributions for crabs in the Meandering and Pulsed plumes experience a sharp decrease in frequency of small, positive heading angles when they are in the upstream section of the plume (Figure 6.4). This indicates that, in the upstream section, crabs in the Meandering and Pulsed plumes cannot determine direction to the plume as accurately as in sections further downstream. Crabs in these more

intermittent plumes seem to determine source direction most accurately in the middle section of the plume, as shown by the increased frequency of small course corrections around the source vector in comparison to more extreme course corrections (Figure 6.3).

6.3 Turning in response to concentration distribution changes

As described in Chapter 3, Section 3.3, plume properties impinging on the upstream leg chemosensors were measured in a sampling box in front of the upstream legs. The goal of this analysis is to test the hypothesis that crabs perform a lateral concentration comparison across this single set of legs, which they then use to determine their course corrections while tracking, moving cross-stream towards the higher stimulus concentration. I determined a concentration center of mass (COM) within this leg-signal analysis box as a measure of the location of the stimulus peak relative to the animal's position, and calculated this parameter at each time step. I compared two measures of the COM (one absolute and one relative) for one second before a turn with the direction of the turn itself (*see* Chapter 3, Section 3.1 for definition of a turn). The mean value of the COM within that period provided an absolute measure of the average direction of concentration bias experienced by crabs. A value of zero indicated a COM along the centerline of the leg analysis box, negative values indicated a mean COM to the left side of the box, and positive values denote the COM was located to the right side of the box.

The analysis revealed that there is a high degree of concordance between the bias of the odor COM and the turn direction. The sign of the mean COM corresponded to the turn direction at least ~70-80 % of the time across the various plume types (Figure 6.5).

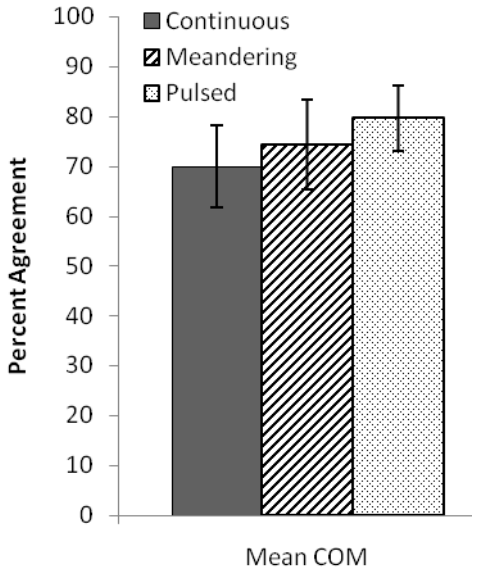

Figure 6.5. Mean percent agreement of the sign of the mean center of mass (COM) of the leg box concentration within one second prior to a turn ± standard error of the mean (SEM) for crabs in various plume types.

This suggests that stimulus bias is a code for direction to tracking animals. It further suggests that COM characterizes the cross-stream, concentration bias shift measured by the legs, and used by the animal to locate itself relative to the plume.

Initial analysis suggested that the distance of that mean shift was often relatively small compared to the size of the leg box in instances where the mean COM did not correlate with subsequent turn direction. Recall that the initial parameterization of the COM is binary (*i.e.* to the left or right of the midline), and that a change in the COM as small as 1 mm (our pixel resolution) would constitute a shift in the present analysis. Because the size of the leg box changed as a function of the angle of the animal and animal size, I explored various thresholds to define a true COM shift by first determining the absolute distances that various thresholds would represent. Thresholds of 2.5 and 5% of the length of the leg box were considered as they were on a scale small enough that COM shifts would be frequent but large enough for the crab to potentially detect. A

2.5% threshold represents a COM movement of roughly ~0.4-0.5 cm withing a single time step whereas a 5% threshold includes movements of approximately ~0.8-0.9 cm within one time step. The size of these leg boxes is significantly affected by plume type (Figure 6.6; $F_{2,74} = 11.53$, $p < 0.001$) as leg boxes of crabs in the Meandering plume are

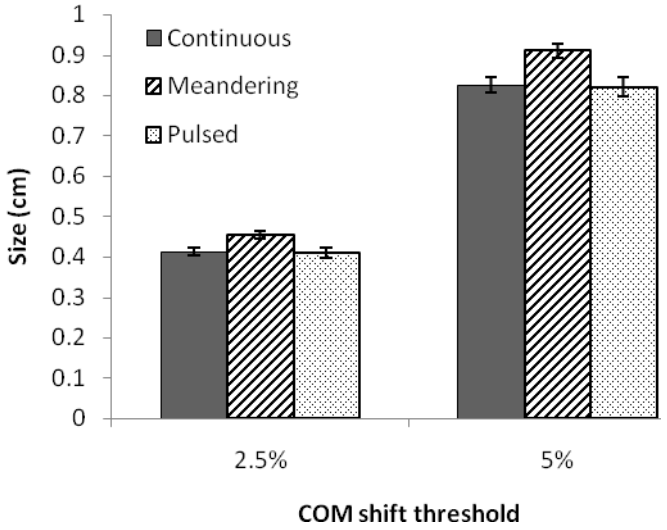

Figure 6.6. Size of a crab leg box relative to the magnitude of the threshold used to determine a shift in COM ± standard error of the mean (SEM) for crabs in various plume types.

larger than leg boxes in the Continuous and Pulsed plumes. The size of the crab is not a significant factor in the size of the leg box as there is no statistically significant difference in the size of crabs used across each plume type (Chapter 3.2.4, $F_{2,36} = 1.63$, $p = 0.21$). There is also a significant effect of threshold applied on the size of the leg box ($F_{1,74} = 111.44$, $p < 0.001$), which is expected because the two thresholds are directly related (*i.e.*, 5% of the size of the leg box is exactly two times greater than 2.5% of the box). There is not a signficant interactive effect of plume type and threshold ($F_{2,74} = 1.28$, $p = 0.28$).

These two different thresholds were then applied to determine whether or not a change in the COM location would be reasonably considered a shift. To observe

instances when it would be advantangeous for the crab to change direction, I examined shift events that occurred in the opposite direction from the direction of the crab's current cross-stream velocity (V_y). Starting from a shift event, I determined the time before the crab turned in the direction of that shift. A turn requires that the y-velocity component changes sign, therefore I defined the actual turn as occuring when $V_y = 0$ cm s^{-1}. I also determined the time that it took the crab to react to the stimulus before making the turn, indicated by when the crab stopped accelerating in its current direction ($A_y = 0$ cm s^{-2}) (Figure 6.7).

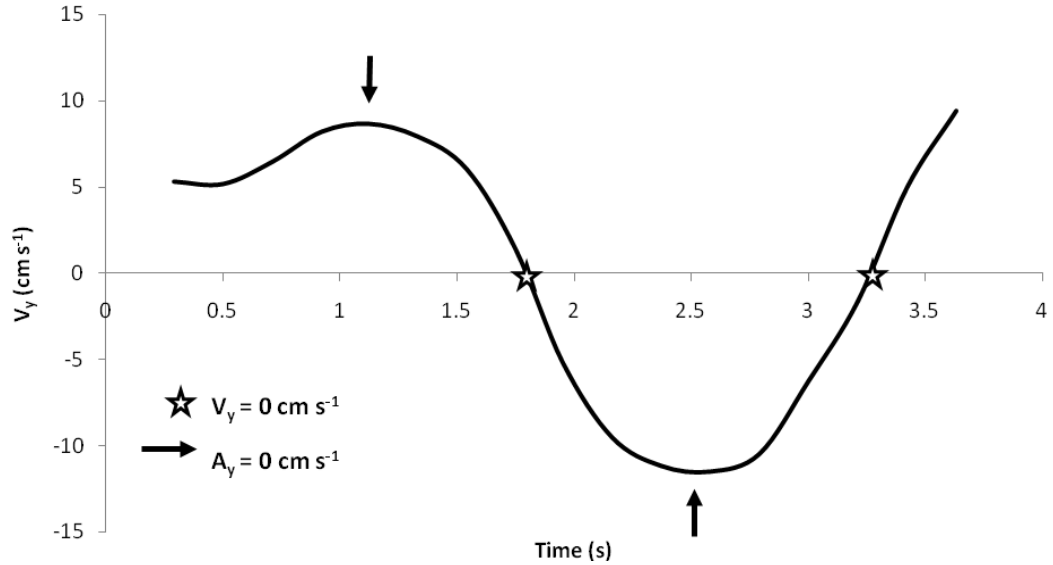

Figure 6.7. Illustration of definition of crab reaction ($A_y = 0$) and direction change ($V_y = 0$) in response to stimuli.

A three-way repeated measures ANOVA was used to examine response time to an antennule spike as a factor of threshold, behavior (*i.e.*, reaction or turn) and plume type (Figure 6.8). I used a repeat measure design as reaction and turn are events recorded from the same individual at two successive times. The analysis revealed that crabs in the Continuous plume both react and change direction significantly more quickly than crabs

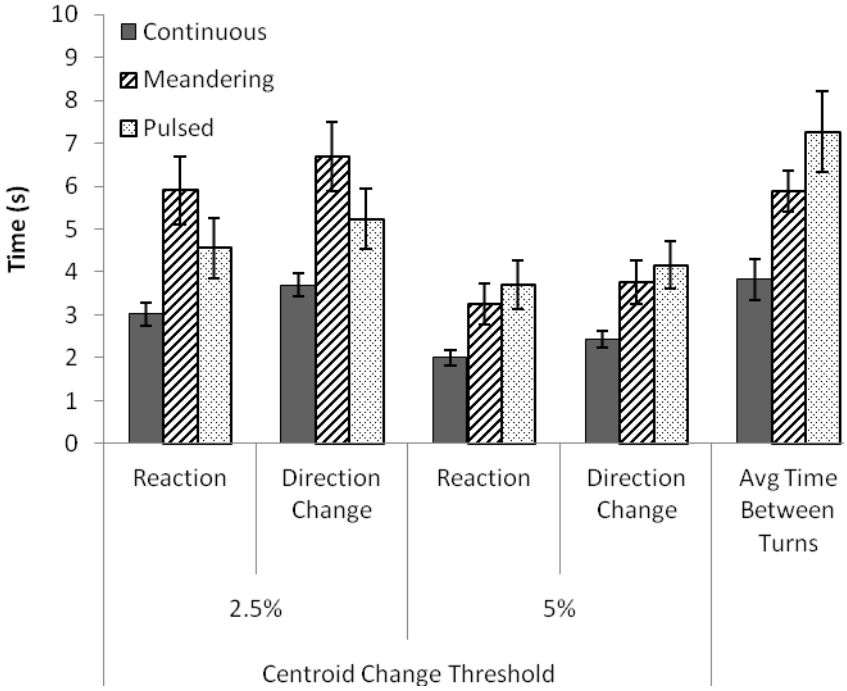

Figure 6.8. Time from a leg box COM shift to a Reaction ($A_y = 0$) and a Direction Change or turn ($V_y = 0$) in the corresponding direction. Data is shown for COM shift thresholds of 2.5% and 5% of the width of the leg box and presented with mean time between turns data for comparison.

in the other two plumes (supported by the significant effect of plume type on response time; $F_{2,322} = 8.24$, $p < 0.001$). This pattern is retained across plume types and thresholds as there is no interaction effect between plume type and reaction/turn times ($F_{2,322} = 2.19$, $p = 0.11$), plume type and threshold ($F_{2,322} = 2.20$, $p = 0.11$), or any three-way interaction of plume type and threshold and reaction/turn time ($F_{2,322} = 1.04$, $p = 0.36$). The time it takes crabs to react is, by definition, always going to be shorter than the time it takes for a crab to make the actual turn, accounting for the significant but trivial effect of behavior ($F_{1,322} = 1346.05$, $p < 0.001$).

A threshold of 5% yeilds response times that are significantly shorter than times observed with a threshold of 2.5% ($F_{1,322} = 13.32$, $p < 0.001$). The response times are less than the mean time observed between turns, suggesting that observed responses are

coincident with COM shifts of 5%. Conversely, a threshold of 2.5% is associated with reaction/turn times that are shorter than the mean time between turns only in the Pulsed plume. In summary, this analysis suggests that a COM shift threshold of 2.5% does not adequately predict turning across all plume types but that a 5% shift is consistently associated with turning.

6.4 Body angle during motion

Previous research has indicated that crabs adjust their body angle to the prevailing flow and odor landscape conditions to balance the conflicting constraints imposed by drag and signal acquisition (Weissburg *et al.* 2003). A low drag posture is favored under conditions of high flow velocity, or in the absence of odor. The crab moves with its legs facing forward and behind (*i.e.*, the rostral-caudal axis is perpendicular to the flow) in this posture, which diminishes odor access and reduces the cross-stream reach of the appendages, restricting cross-stream comparisons of odor bias. Because contribution of drag to crab body angle is standardized across all plume types (free-stream velocity = 5 cm s^{-1}), body angle provides a way to evaluate the relative difficulty crabs face when tracking each plume type. I would expect the body angle to become more perpendicular (90 degrees) to the flow with increased need for antennule signal contact and leg signal contrast corresponding to increased plume tracking difficulty. I hypothesized that crabs may change their body angle in response to their direction of cross-stream movement (positive vs. negative V_y) to maximize signal reception. I additionally hypothesized that crabs that receive a concentration spike may respond by temporarily decreasing their angle to flow to move upstream with minimized drag, and increase their angle to flow when they lack adequate signal to govern tracking.

Normal statistical methods are not applicable to circular values and, while basic analysis of variance tests (ANOVA) have been developed, there is no repeated measure test available for these type of data. Consequently, I used ANOVA's specific for circular data (Harrison *et al.* 1986) to compare body angles and rotational velocities within each plume type for each treatment.

Plume type significantly affects the body angle of the crab over the entire path (Fig 6.9, Total Path; $F_{2,37} = 8.08$, $p = 0.001$) as crabs in the Meandering plume

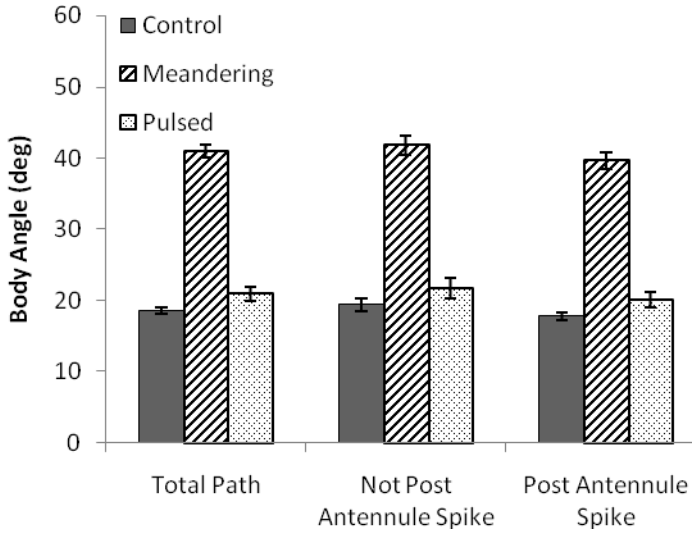

Figure 6.9. Mean crab body angle in relation to flow direction (longest body axis parallel to flow = 0˚; longest body axis perpendicular to flow = 90˚) ± standard error of the mean (SEM) for crabs in various plume types. Mean body angle is displayed for the Total Path and for crabs one second following (Post) receiving an odorant spike at the antennules and crabs that have not received an odor spike (Not Post) within the previous one second.

consistently move at body angles roughly twice as large (more perpendicular to flow, than the body angles of crabs in the Continuous and Pulsed plumes, which track at similar body angles. Crab body angle is not significantly affected by receiving a spike at the antennules (Continuous: $F_{1,28} = 0.13$, $p = 0.72$; Meandering: $F_{1,24} = 0.02$, $p = 0.90$; Pulsed: $F_{1,23} = 0.78$, $p = 0.39$).

There is a significant effect of cross-stream direction of movement on the body angle of crabs in all plume types when crabs are walking to the left versus the right (Figure 6.10; Continuous: $F_{1,28} = 4.98$, $p = 0.03$; Meandering: $F_{1,24} = 4.56$, $p = 0.04$;

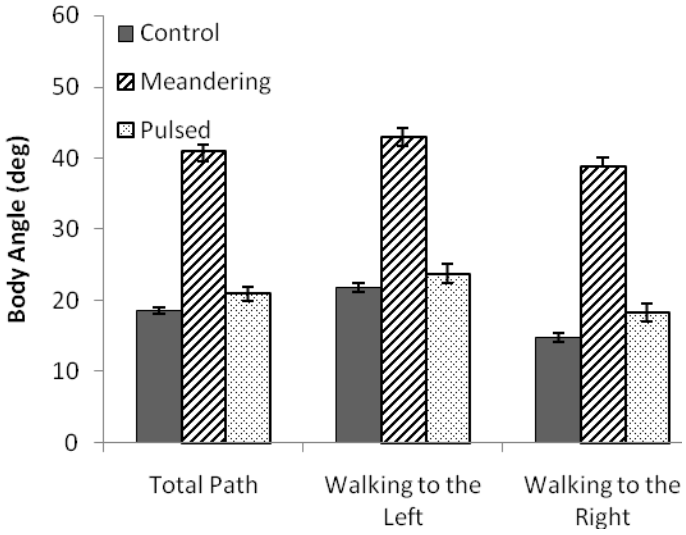

Figure 6.10. Mean crab body angle in relation to flow direction (longest body axis parallel to flow = 0˚; longest body axis perpendicular to flow = 90˚) ± standard error of the mean (SEM) for crabs in various plume types. Mean body angle is displayed for the Total Path and for crabs moving cross-stream to the left (Negative V_y) or right (Positive V_y).

Pulsed: $F_{1,22} = 5.33$, $p = 0.03$). In each case, there is a slight increase in body angle when crabs move to the left (negative cross-stream velocity), indicating that crabs preferentially make a very slight body angle correction towards their direction of movement. Crabs moving cross-stream towards the centerline do not move at a significantly different body angle compared to crabs moving cross-stream away from the centerline (Figure 6.11; Continuous: $F_{1,28} = 0.013$, $p = 0.91$; Meandering: $F_{1,24} = 0.15$, $p = 0.70$; Pulsed: $F_{1,22} = 0.16$, $p = 0.69$).

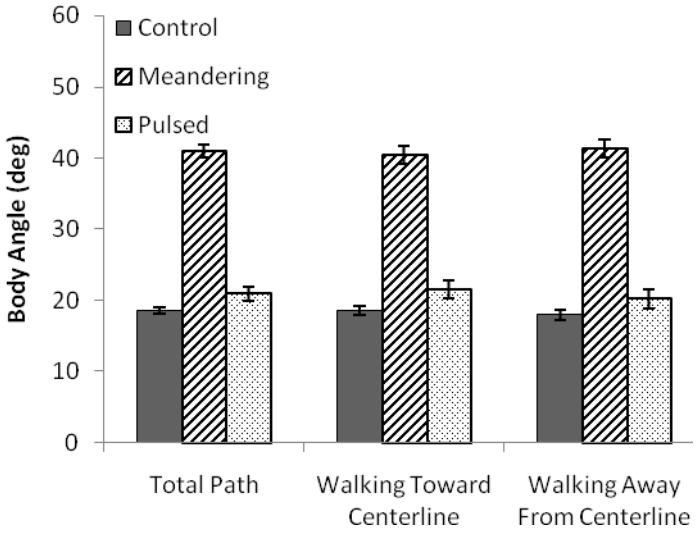

Figure 6.11. Mean crab body angle in relation to flow direction (longest body axis parallel to flow = 0˚; longest body axis perpendicular to flow = 90˚) ± standard error of the mean (SEM) for crabs in various plume types. Mean body angle is displayed for the Total Path and for crabs moving Towards the Centerline and Away from the Centerline.

Due to the significant effect of left-right cross-stream velocity on the body angle of crabs over the entire plume I hypothesized that the observed differences may arise from crabs rotating their body with respect to flow in the process of turning (characterized as a sign change in cross-stream velocity, V_y). I analyzed the body angle of crabs immediately (one second) after turns of different directions across different plume types. There was no significant difference between body angles of crabs immediately after turns to the left or the right (Figure 6.12; Continuous: $F_{1,25} = 0.09$, $p = 0.77$; Meandering: $F_{1,20} = 0.06$, $p = 0.81$; Pulsed: $F_{1,18} = 1.01$, $p = 0.33$). Neither do crabs show any significant body angle change after they turn towards the centerline or away from the centerline (Figure 6.13; Continuous: $F_{1,19} = 0.31$, $p = 0.0.59$; Meandering: $F_{1,16} = 0.07$, $p = 0.80$; Pulsed: $F_{1,13} = 0.006$, $p = 0.94$). This suggests that crabs do not change their body angle when making a turn. However, due to the variation in body angle across a crab's path, analyzing mean body angle across an entire path and across all crabs in a

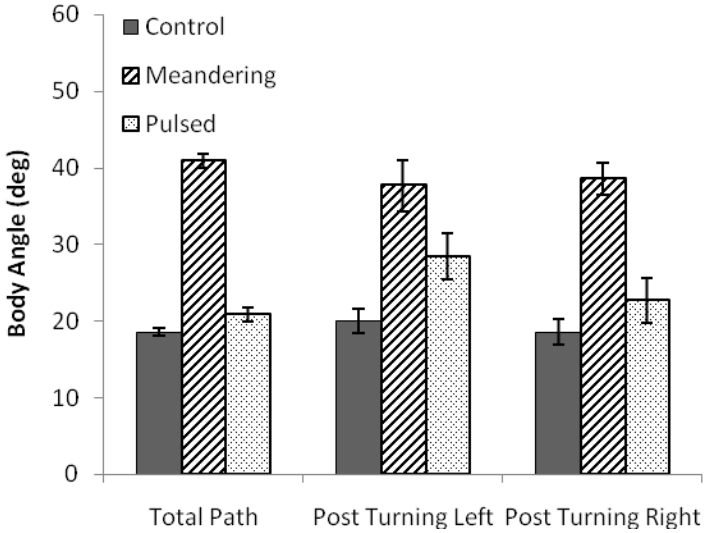

Figure 6.12. Mean crab body angle in relation to flow direction (longest body axis parallel to flow = 0˚; longest body axis perpendicular to flow = 90˚) ± standard error of the mean (SEM) for crabs in various plume types. Mean body angle is displayed for the Total Path and for crabs in the one second after (Post) turning to the Left or Right.

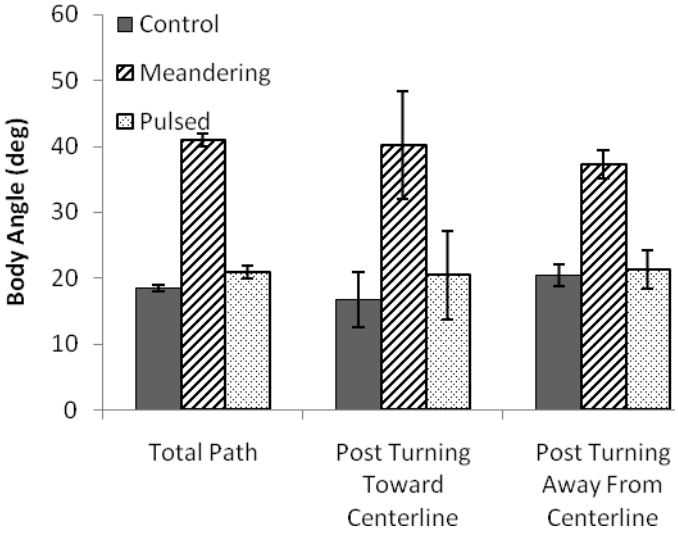

Figure 6.13. Mean crab body angle in relation to flow direction (longest body axis parallel to flow = 0˚; longest body axis perpendicular to flow = 90˚) ± standard error of the mean (SEM) for crabs in various plume types. Mean body angle is displayed for the Total Path and for crabs in the one second after (Post) making a turn Towards the Centerline or Away from the Centerline.

single plume may not be sensitive enough to determine if crabs are changing their body angle in association with a turn.

6.5 Rotational velocity

Change in the body angle of a crab with time can be measured by rotational velocity or rotational acceleration, where the rotation is measured around the center of mass of the crab. This measure of crab rotation may be more sensitive to rotational responses to odor as it does not rely on the absolute value of the crab's body angle. Preliminary analysis revealed that statistical significance cannot be determined for the mean path rotational acceleration of tracking crabs because the standardized magnitude of a crab's rotational acceleration is smaller than what can validly be tested statistically (Zar 1998), thus preventing further discussion.

The mean rotational velocity of tracking crabs is not significantly affected by plume type over the entire path (Figure 6.14, Total Path; $F_{2,37} = 0.10$, $p = 0.90$).

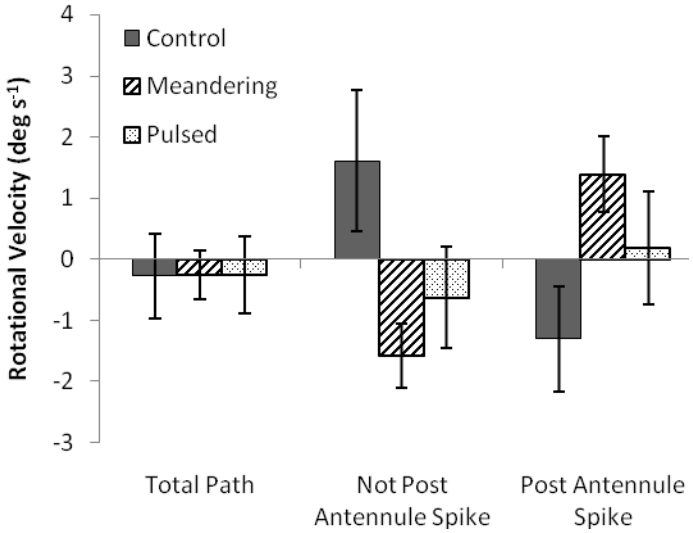

Figure 6.14. Average rotational velocity of the crab's body around its Center of Mass (COM) ± standard error of the mean (SEM) for crabs in various plume types. Mean rotational velocity is displayed for the Total Path and for crabs one second following (Post) receiving an odor spike at the antennules and crabs that have not received an odor spike (NonPost) within the previous one second.

However, crabs in the Continuous and Meandering plumes do have significantly different rotational velocities depending on whether they have received a spike within the past one second (Continuous: $F_{1,28} = 5.44$, $p = 0.03$; Meandering: $F_{1,24} = 5.05$, $p = 0.03$). Crabs that receive a spike in the Continuous plume rotate to face the plume more directly (positive rotation) and those that have not received a spike rotate their body to be more parallel with the mean flow direction (negative rotation). Interestingly the converse occurs with crabs in the Meandering plume: crabs that receive a spike rotate their body to be more in line with the mean flow vector, while those crabs that have not received a spike rotate their body to face the plume more directly. The rotational velocity of crabs in the Pulsed plume appears unaffected by receiving a spike ($F_{1,22} = 0.17$, $p = 0.68$).

A crab's rotational velocity is significantly affected by its cross-stream direction of movement in the left-right orientation (Figure 6.15; Continuous: $F_{1,28} = 20.44$, $p = 0.0001$; Meandering: $F_{1,24} = 9.35$, $p = 0.005$; Pulsed: $F_{1,22} = 5.03$, $p = 0.04$), but not in the orientation towards-away from the centerline in the Continuous or Meandering plumes (Figure 6.16; Continuous: $F_{1,28} = 0.18$, $p = 0.68$; Meandering: $F_{1,24} = 0.11$, $p = 0.75$). The rotational velocity of crabs in the Pulsed plume was significantly affected by whether they were moving towards the centerline or away from the centerline ($F_{1,22} = 4.51$, $p = 0.05$). The average rotational velocity of crabs after turns was not significantly affected by the direction of the turn (left-right: Figure 6.17; toward-away: Figure 6.18; $F \leq 1.48$, $p \geq 0.33$ across all analyses).

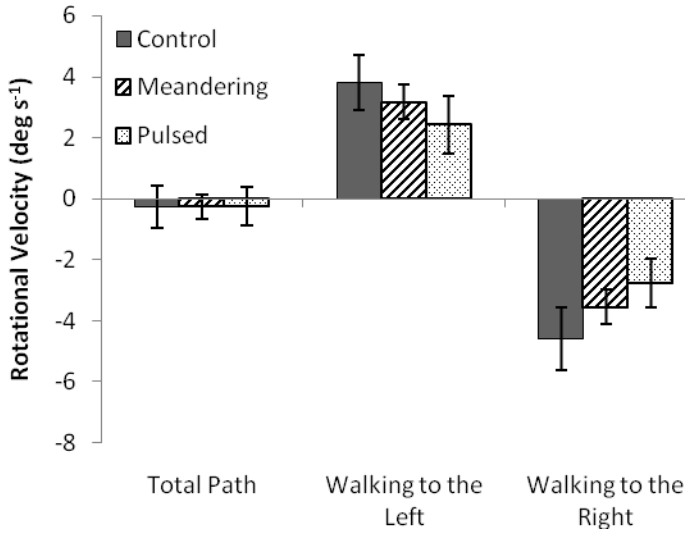

Figure 6.15. Average rotational velocity of the crab's body around its Center of Mass (COM) ± standard error of the mean (SEM) for crabs in various plume types. Mean rotational velocity is displayed for the Total Path and for crabs moving cross-stream to the right (Positive V_y) or left (Negative V_y).

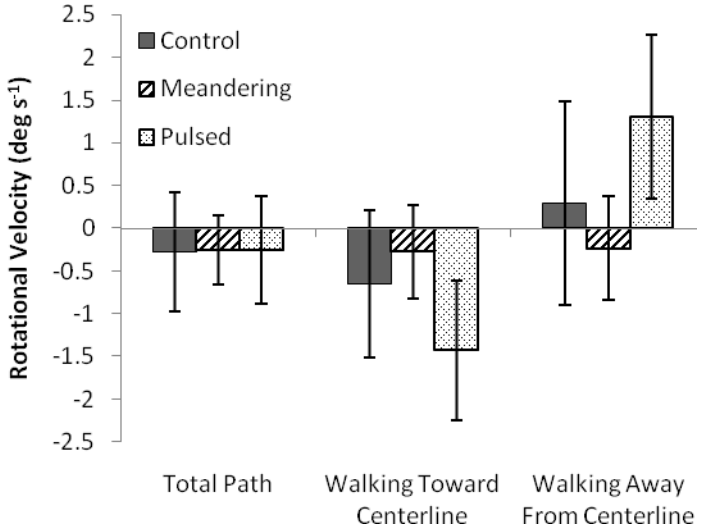

Figure 6.16. Average rotational velocity of the crab's body around its Center of Mass (COM) ± standard error of the mean (SEM) for crabs in various plume types. Mean rotational velocity is displayed for the Total Path and for crabs moving Towards the Centerline and Away from the Centerline.

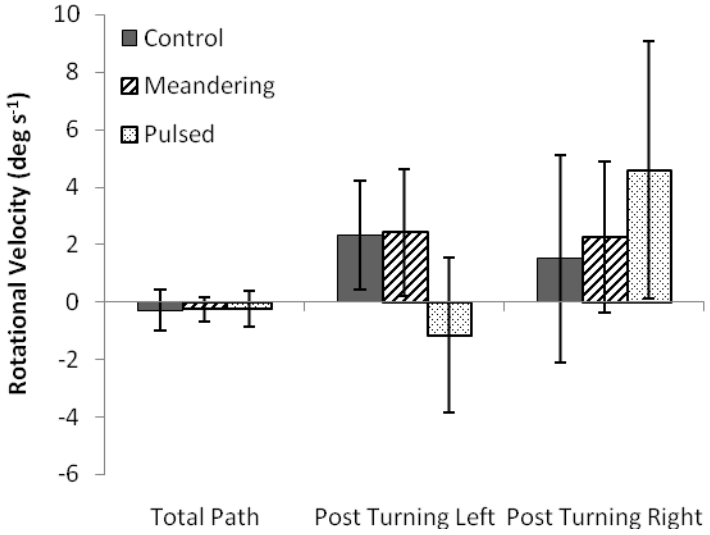

Figure 6.17. Average rotational velocity of the crab's body around its Center of Mass (COM) ± standard error of the mean (SEM) for crabs in various plume types. Mean body angle is displayed for the Total Path and for crabs in the one second after (Post) turning to the Left or Right.

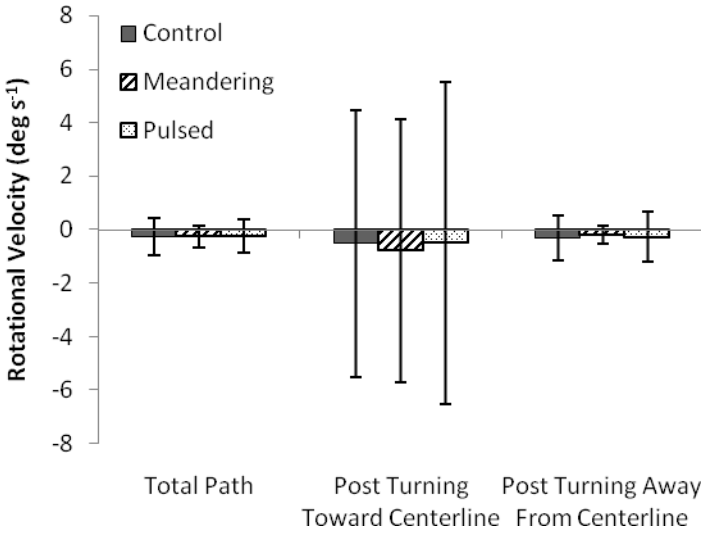

Figure 6.18. Average rotational velocity of the crab's body around its Center of Mass (COM) ± standard error of the mean (SEM) for crabs in various plume types. Mean body angle is displayed for the Total Path and for crabs in the one second after (Post) making a turn Towards the Centerline or Away from the Centerline.

6.6 Cross-stream motion data summary

6.6.1 Course corrections towards the plume centerline

Analysis of cross-stream patterns of movement in tracking blue crabs reveals a remarkable sensitivity on the part of the crabs to the source direction. Crabs frequently make a small, positive course corrections in their heading angle as they move upstream over the entire track (Figure 6.1) and within each section (Figs. 6.2, 6.3, and 6.4), regardless of plume type. Crabs moving downstream ($\alpha < -90^{\circ}$ and $\alpha > 90^{\circ}$) in the Meandering and Pulsed plumes are also more likely to head towards the plume centerline, shown by the greater frequency of heading angles larger than 90˚ (Figure 6.1). There is a pronounced local maxima in the Meandering profile around -90˚ which is absent in the Continuous profile and barely indicated in the Pulsed plume. Heading angles of roughly -90˚ indicate crabs that are moving almost completely cross-stream away from the center of the plume. This suggests that crabs in the Meandering plume are following the plume Meander as it moves to the side, even when it is not towards the plume centerline. The local maxima at 90˚ for all plume types indicate that crabs use spatial contrasts in chemical signal intensity to orient to the source. While crabs in the Meandering plumes may be following the meander as it shifts towards the plume centerline, crabs in the Continuous and Pulsed plume are able to compare signals across multiple sensors in space and sharply correct their path when signal contrast indicates that they are moving out of the plume.

Heading angle distributions suggest that crabs in the Meandering plume follow the plume Meander even when it is not aligned with the mean flow vector or headed towards the plume centerline. While it is extremely rare for crabs in the downstream

section of the plume (Figure 6.2) to make negative course corrections less that -90˚, crabs in the Meandering plume show a pronounced peak at -90˚, indicating that they are the most likely of the three plume types to make course corrections that are directly away from the source vector (negative corrections).

Crabs in the Pulsed plume have a greater tendency towards large course corrections in both positive and negative directions in the middle section of the plume (Figure 6.3) and their percent of small course corrections is smaller than for crabs in the Continuous or Meandering plumes. The fundamental difference between the Pulsed plumes and the other two plumes is the temporal intermittency, which suggests that the extreme course corrections seem to be an attempt to regain plume contact during the inevitable loss of contact with the plume in between pulses. Crabs in the Pulsed plume move downstream more frequently than crabs in the other plumes in the middle section as well but have a tendency towards positive angles over negative angles when they are moving backwards. This suggests that crabs in the Pulsed plume move towards the plume centerline (towards higher concentration) when they do move backwards (against the flow), which supports the idea that concentration parameters can override the influence of the mean flow, similar to the mechanisms driving large, cross-stream course corrections in the Meandering plume.

The frequency distributions of heading angles in the upstream section of the plume are the least uniform in shape, characterized by low frequencies (due to the shortened time records in this section) and many local maxima (Figure 6.4). Crabs in this section of the plume that do move backwards do so almost exclusively towards the plume

centerline. Small course corrections still trend towards positive angles but large course corrections are more frequently negative.

6.6.2 Spatial resolution related to leg box width and body angle

Because crab body angles in the Continuous and Pulsed plumes are very low with respect to the mean flow (~20˚; Figure 6.9) this indicates that crabs may have the ability to compare signal contrast between individual legs to mediate position within the plume rather than bilaterally across left and right leg sets according to previous hypotheses. Although such low angles may correspond to smaller transverse spans of the animal than if they were oriented perpendicular to the flow, the group of legs on the upstream side of the body will still sample in the transverse direction and permit animals to detect odor stimulus asymmetry across the flow. This ability is highly beneficial for tracking crabs as it allows the crabs to determine stimulus asymmetry without sacrificing drag by rotating their body so they face into the flow, which would be necessary for detecting transverse stimulus asymmetry across the main body axis. This indicates that we should expect crabs to see crabs sacrifice drag in order to take advantage of stimulus asymmetry across their body when they are trying to make the widest cross-stream stimulus comparisons. Crabs that do not need such wide comparisons should reduce drag by aligning their body more closely with the mean flow vector, which will allow the widest possible comparison with a single set of legs even though the leg box width will be the smallest.

The body angles of the crabs seem to support this prediction: crabs that are tracking a plume with great spatial intermittency (Meandering) face more directly into the flow than crabs that are not faced with great spatial intermittency (Continuous and

Pulsed) (Figure 6.8). The leg box sizes directly corroborate this data as crabs in the Meandering plume have larger leg boxes than crabs in the other two plume types (Figure 6.6), which is expected as the leg box will increase in size in relation to increasing body angle up to a maximum width at 90˚.

6.6.3 Course corrections in response to plume concentration changes

The frequent course corrections towards the source vector are remarkable and this indicates that crabs are extremely sensitive to their position within the plume. Measuring the center of mass (COM) of the concentration impinging upon the upstream leg sensors at any given time confirmed that the direction that crabs decide to turn corresponds with the mean direction of the COM shift at least 70% of the time across all plume types (Figure 6.5), even before a movement threshold was applied. We assume that there is some lower threshold where crabs would be unable to sense extremely small COM shifts based on the physical separation of the upstream leg sensors and that those small shifts are relatively random and not indicative of plume source location. We also know that crabs seem remarkably adept at adjusting their position within the plume (Figs. 6.1-6.4), and therefore must be very sensitive to COM shifts in order to respond so rapidly and accurately to its presumed direction of the plume source. Applying a movement threshold of 5% of the leg box width corresponds to roughly a 0.8-0.9 cm cross-stream COM shift (Figure 6.8), and incorporates the acute sensing abilities of the crab with the size of the COM shift that is potentially useful as spatial information. This measure demonstrates that crabs can determine cross-stream directional shifts of a turbulent plume of less than a centimeter and use those shifts to accurately navigate the plume to its source.

Crabs in the Continuous plume are the quickest to react to a concentration shift (~2 s) while crabs in the Meandering and Pulsed plumes take longer to react (~3.5-4 s). This reaction time may be due to the perceived directional quality of the information present in that directional shift. For crabs in the Continuous plume, cross-stream changes in concentration are a more reliable indicator of the direction to the plume centerline than in the Meandering plume (Dickman 2008). Crabs in the Continuous plume also receive more frequent (Figure 5.1) and more concentrated signals (Figure 4.1) than crabs in the Pulsed plume.

The frequent cross-stream shifts in the plume concentration COM may cause crabs in the Meandering plume to shift into a different state where they wait for multiple COM shifts across its upstream legs or wait a certain amount of time to ensure they do not get any conflicting information before moving in the direction of the COM shift. Crabs in the Pulsed plume frequently lose signal, even when correctly following a COM shift, and experience lower filament concentrations than the other plumes due to homogenization. These signals may again cause a state shift, as crabs that follow COM shifts will frequently exit the plume, thereby indicating to the crab that COM shifts are not a reliable indicator of the plume centerline, as seen in the Meandering plume. The lower concentration of filaments may additionally cause the crab to wait longer after a COM shift to ensure correct interpretation of the COM direction. Regardless of the time to the reaction after a COM shift, the time between crabs reacting to a COM shift ($A_y = 0$) and the crab actually making the turn ($V_y = 0$) is very short (< 0.5 s), indicating these two events are nearly coincident.

6.6.4 Crab body angle and rotational velocity while tracking

6.6.4.1 Crab response to antennule concentration spikes

While the absolute body angle of crabs does not seem to differ whether a crab received an antennule spike or not (Figure 6.9), reception of an antennule spike does seem to affect the rotational velocity of crabs in the Continuous and Meandering plumes, but not the Pulsed plume (Figure 6.14). What is very interesting is that crabs in the Continuous plume rotate their body more parallel with flow when they receive an antennule spike, while the crabs in the Meandering plume rotate their body so they face the flow in response to an antennule spike. This may have something to do with their absolute body angle and the characteristics of the plume that they are tracking. Crabs in the Continuous plume track at shallow angles (Figure 6.8) and receive spikes more frequently than crabs in the Meandering plume (Figure 5.1). We know that crabs must balance the drag associated with a higher body angle to the flow with signal acquisition (Weissburg *et al.* 2003), so crabs may be using a state-dependent search algorithm to control their body angle in relation to the flow. Crabs that are receiving a more frequent signal may be able to afford to reduce drag and therefore move upstream more quickly or with less energy. Conversely, crabs that are not receiving frequent signals may react to receiving an antennule spike by increasing their drag in order to increase their signal perception ability and take advantage of the "rare" information. Crabs in the Meandering and Pulsed plumes receive spikes at similar frequencies (Figure 5.1) so we would expect their patterns to be the same if rotational velocity to spike reception was based only on frequency, and yet spike reception does not play a significant role in the rotational velocity of crabs in the Pulsed plume. The data suggest that concentration plays a role in

this process, as crabs in the Pulsed plume receive much less concentrated spikes than crabs in the Continuous or Meandering plumes (Figure 4.1). The weak and infrequent information received by crabs in the Pulsed plume indicates that crabs may only respond to an antennule spike by rotating their body if the spike is above some concentration threshold.

6.6.4.2 Crab response to cross-stream movement to the left and right

The body angle (Figure 6.10) and the rotational velocity (Figure 6.15) of crabs while they are moving cross-stream to the left or right suggest that crabs bias their body angle slightly in the direction of their primary movement. Crabs moving towards the laser (V_y = positive) are facing in the direction of their movement. In these cases the crabs tend towards lower body angles that are more in line with the prevailing flow direction. Crabs that move away from the laser (V_y = negative; backwards) do so at steeper body angles to the flow than those moving towards the laser. This indicates that a crab's body angle while tracking in these three plume types is relatively consistent, but that crabs preferentially rotate slightly in the direction that they are moving.

Crabs in the Continuous plume tend to rotate their bodies in the direction of their movement most rapidly, followed by crabs in the Meandering and then Pulsed plumes (Figure 6.15). This may be a factor of the reliability of signals as indications of source direction. Similar to COM reaction time (Figure 6.8, discussed in 6.6.3), crabs that are receiving less frequent and/or concentrated signals may not react as quickly to stimuli or make any otherwise rapid movements (including rotation), since these responses may not result in turns towards the source.

These changes in body angle and rotational velocity do not happen in association with the turns themselves (Angle: Figure 6.12; velocity: Figure 6.17), suggesting that crabs rotate their body angle after they have already started moving in a particular direction. As discussed in the introduction, blue crabs are not particularly visual predators but they do have some visual capacity that allows form and motion discrimination over short distances. This body angle change in the direction of motion may be an attempt to increase visual ability in that direction. Alternately, the rotated body angle may be a result of the direction of movement itself as the coordination of leg movement for the crab to move in a particular direction tends to skew the body angle preferentially in that direction.

6.6.4.3 Crab response to cross-stream movement in relation to the centerline

Though the body angle and rotational velocity of crabs is correlated with left and right cross-stream movement, they are generally not related to cross-stream movement in relation to the plume centerline (Angle: Figure 6.11; Rotational Velocity: Figure 6.16). This again bolsters the argument that the left-right differences in body angle are a function of direction of travel. What is salient is that crabs in Pulsed plumes are the one instance in which there is a significant relationship between movement towards or away from the centerline and the rotational velocity. When they are walking toward the centerline, crabs in Pulsed plumes rotate their bodies to a shallower angle more in line with the mean flow direction, and while walking away from the centerline these crabs rotate their bodies so they face the flow more directly. Due to the relative weak concentration of the signals received by crabs in the Pulsed plume (Figure 4.1), these crabs may rely on other factors associated with heading towards the plume centerline

(*e.g.*, increased intermittency) as indicators of whether they can afford to reduce drag and move upstream more quickly or whether they should increase drag and assess the signal more thoroughly. This pattern of behavior would be analogous to the behavior we see in Continuous crabs in response to an antennule spike (Figure 6.14). Crabs receiving a positive signal (i.e., frequent and concentrated spikes or indication of heading towards the plume centerline) can afford to decrease their drag as they move upstream; crabs receiving a negative signal (i.e., less frequent, concentrated spikes or indication of heading away from the plume centerline) increase their drag to better assess available signals.

CHAPTER 7

DISCUSSION AND CONCLUSIONS

7.1 Dissertation goals

In this dissertation, I examined chemically mediated tracking behavior in fluid environments by using *Callinectes sapidus,* the blue crab, as my model organism. Specifically, I examined *C. sapidus* tracking behavior in conjunction with laser-induced fluorescence (LIF) measurements of odorant plume structure to investigate how turbulence affects the three-dimensional structure of odorant plumes and subsequently mediates olfactory search efficiency and success (Chapter 2). Additionally, I participated in a multi-disciplinary effort to develop a novel, three-dimensional, simultaneous sampling system to sample odorant plume structure concurrently with quantification of blue crab tracking behavior (Chapter 3). This 3DLIF system was instrumental in determining the true 3D nature of odorant signals experienced by a tracking organism, which is critical to resolve the specific odor plume properties that mediate successful olfactory navigation within a plume. The increased understanding of the basic features of the signal environment and the animal responses themselves (Chapter 4) laid the groundwork for in-depth analysis of my experimental data obtained with the 3DLIF-behavior visualization system, which tested hypothesis of the specific plume properties mediating the along-stream (Chapter 5) and cross-stream (Chapter 6) components of a successful track.

The findings from this dissertation greatly contribute to our understanding of odorant signal structure, stimulus integration by tracking organisms, and the resulting

chemosensory navigation strategies. Effectively using odorant structure to navigate chemical plumes is a common problem for a wide range of organisms (*e.g.*, bacteria: Berg and Anderson 1973; ants: Brun 1914; moths: Mafra-Neto and Cardé 1995; whelks: Ferner and Weissburg 2005; lobsters: Devine and Atema 1982). Even humans are faced with this problem as we attempt to modify the search strategies of autonomous agents so they are better able to follow chemical plumes to their source. On a similar note, the data in this dissertation provides an opportunity to make a direct connection between signal properties and resulting behavior, thereby giving us information about how crabs process multiple, and perhaps conflicting (*e.g.*, hydrodynamic vs. chemical) signals. These types of connections directly contribute to a greater understanding of how stimuli are integrated by a crab's nervous system to produce certain behaviors. We can then not only draw parallels between the nervous system organization and function in blue crabs and that of other decapods, we can additionally make connections to the olfactory processing of insects due to similarities between the olfactory pathways of decapods and insects (Schmidt 2007). Because chemical stimuli are so ubiquitous and routinely utilized by organisms to mediate their inter- and intraspecies interactions (Dusenbery 1992; Zimmer and Butman 2000), understanding animal responses to these stimuli will provide information on a wide range of interactions that have the potential to alter community structure and composition (Dill 1987; Raimondi *et al.* 2000; Turner *et al.* 2000; Trussell *et al.* 2003).

7.2 From signal to behavior

In order to understand the patterns of behavior that are in some way modulated by chemosensory information, we must understand all the events that occur between, and

inclusive of, the initial stimulus production and the final behavioral output. To achieve a definition of a true signal that is utilized by a particular animal requires integration of the nature of the stimulus (*e.g.*, magnitude, spatial and temporal dynamics), its physiological effects (*e.g.*, transduction by receptors, generation of neural response, integration in central nervous system), and the resulting behavioral output. Understanding what constitutes a behavioral signal is essential to provide a framework from which to better understand how neural circuits generate these behaviors and what types of behaviors are actively facilitating the acquisition and perception of these functionally important signals.

7.2.1 What constitutes a signal?

The first step in understanding the signaling cascade that leads to chemosensory related behavior is defining the nature of the stimulus. Chemical cues have two major characteristics: their quantity and their quality. Odorant quality is generally taken to mean the identity of the chemical as delineated by its molecular structure, whereas quantity is indicated by the intensity or concentration, of the signals. An organism may perceive an odorant attractive at low intensities, yet perceive the same odorant as repulsive at higher intensities. For example, *Drosophila mellanogaster* are attracted to traps baited with intermediate concentrations of the odorant 1-Octen-3-ol but repelled from traps with a high concentration of the odorant (Chow and Frye 2009).

7.2.1.1 Coding olfactory stimuli

This problem of coding the different aspects of olfactory stimuli is particularly interesting because odorants have discrete structures and a variety of physicochemical properties that do not fall along a continuous function such as auditory or visual stimuli (*i.e.*, wavelength or frequency of sound or light). An organism's perception of odorant

quality can be the result of an odorant interacting with a multitude of olfactory receptor neurons (ORNs), which in turn can interact with many odorants (Malnic *et al.* 1999). The combination of activated ORNs produces a coding scheme of action potentials, which reflects the features of a variety of specific odorants. This coding scheme was first recognized in vertebrates and a similar coding schemes have been determined for insects (*e.g.*, *Drosophila:* Hallem and Carlson 2006) and crustaceans (*see* Derby *et al.* 2001), indicating that this mechanism for identifying odorants is at least partially utilized by invertebrates as well. In *Drosophila,* individual odorant receptors span a continuum from narrowly tuned to broadly tuned, with the broadly tuned receptors showing strong excitatory responses to many odorants but are most sensitive to odorants that have structural similarities (Hallem and Carlson 2006). In particular, the temporal dynamics of the receptor response patterns provide extensive information about the quality, quantity, and the duration of a particular odorant.

Unfortunately, the particular odorants that drive chemosensory search in the blue crab are not yet known, though there is research demonstrating crabs are not responsive to a suite of amino acids mimicking the composition of those found in injured prey (Finelli *et al.* 2000). This in itself is particularly interesting because a variety of other crustaceans are attracted to these amino acid mixtures (hermit crabs: Rittschof 1980; lobster: Carr and Derby 1986; shrimp: Daniel and Bayer 1987). In addition, physiological investigations have show that crustaceans are capable of detecting individual amino acids and amino acid mixtures (Derby and Atema 1982; Johnson and Ache 1982), though these physiological measurements have not been performed on blue crabs in particular.

Although this study does not elucidate the molecular identity of signals that evoke tracking, it has increased our understanding about the relative intensity of odorant experienced by tracking crabs and the spatial and temporal characteristics of these signals. The findings from the bed roughness studies in Chapter 2 support the hypothesis that the upstream motion of a tracking crab is governed by large instantaneous concentration bursts at the antennules. Walking speed (Figure 2.1b) and tracking success (Table 2.2) decrease with increased bed roughness as the odorant intensity correspondingly decreases as a result of plume homogenization. There is also a marked decline in tracking performance with decreased source concentration over the same bed roughness (Table 2.2), indicative of a relationship between the intensity of odorant bursts and the upstream movement of a tracking crab. I hypothesized that the intensity and the intermittence of the odorant bursts received at a crab's antennules would both have direct bearing on resultant upstream velocity; in particular, the decrease in filament concentration and intermittency caused by the increase in bed roughness leads to a decreased probability of encountering concentrated odor filaments, thereby causing slower progression towards the source. Sensory neurons of all types adapt to particular stimuli (*e.g.,* spider mechanoreceptors: Juusola and French 1998; mice olfactory receptors: Boccaccio *et al.* 2006; turtle auditory hair cells: Ricci *et al.* 2000; finch optical receptors: Schmidt and Bischof 2001), that is, neurons become less responsive over time to a relatively constant stimulus. For this reason, I anticipated that the change in stimulus concentration may be more stimulatory for tracking blue crabs than the absolute concentration of individual filaments.

7.2.1.2 Stimulus concentration

The results from the 3DLIF studies suggests that tracking crabs do not react to increasing stimulus concentration with a gradient of behaviors directly relating the response intensity to the magnitude or the degree of change in stimulus concentration (Figs. 5.15-5.17). Blue crabs do not directly respond to absolute changes in spike concentration (Figure 5.15) or changes in spike concentration with time (Figs. 5.16 and 5.17). Instead, crabs appear to respond to stimulus concentration changes with a simple step function, whereby concentrations over a certain threshold elicit behavior. This step function is then augmented by a series of state changes, which alter reactions to subsequent stimuli. These state changes may result in graded changes in behavior that superficially appear unrelated to the magnitude of a stimuli, yet may be still be indirectly related to concentration (*e.g.,* intermittency).

It seems clear that crabs in these studies are responding to an odorant once concentration exceeds a certain threshold, yet it is difficult to resolve the precise threshold without knowing the identity of the odorant. However, the time records of stimulus intensity patterns arriving at receptor organs give indications of stimulation levels necessary to ensure a high level of contrast between background odorant and relevant spikes. The criterion used was that the threshold should be above the standard deviation of concentration fluctuations within the plume order to be sufficiently resolvable. Although the choice of the threshold criterion used in subsequent analysis ($AvgC_{Amax}/2$) as behaviorally salient is not based on direct physiological data, the analysis using this threshold suggests in generally predicts or explains the responses of animals in plumes. Putative behaviorally relevant spikes of concentration are on average

often two orders of magnitude greater than the mean concentration of the plume, and an order of magnitude greater than the other minor peaks in concentration. While blue crabs respond to with a threshold function to changes in concentration, this does not necessarily imply that graded responses to chemical concentration are unimportant. For instance, Zimmer-Faust *et al.* (1996) demonstrated that spiny lobsters have a state dependent reaction to stimulus concentration, where active lobsters respond to a stimulus (ATP) concentration two orders of magnitude smaller than necessary to evoke a response in resting lobsters. Resting blue crabs may be similarly responsive to greatly increased odorant concentrations, which could be indicative a large or very close source of food. Blue crabs may also utilize the concentration of conflicting odorants as a measure of risk assessment (*e.g.*, Moir and Weissburg 2009).

Prior research on what constitutes an odorant stimulus to a decapod crustacean has operated largely at a physiological level, examining the pulse duration and concentration that stimulates individual chemosensor cells. In particular, Gomez *et al.* (1999) demonstrated that, after an initial spike reaction based on concentration, chemosensor responses to subsequent bursts (2 Hz) were completely indistinguishable across a threefold stimulant concentration range (10 μM to 1000 μM concentration of hydroxoproline [Hyp]). At a stimulus rate of 0.5 Hz, the higher concentrations were indistinguishable in their reaction after the first pulse, while chemosensors were significantly less responsive to the lowest concentration of stimuli (10 μM). Gomez and Atema (1996) previously demonstrated that for short stimulus bursts (*i.e.*, less than 100 ms) Hyp concentrations less than 10 μM failed to elicit a physiological response from the chemoreceptor cells yet did stimulate the cells by 200 ms. Additionally, these cells had

the greatest stimulus concentration discrimination at ~200 ms. These data clearly indicate that crustacean (lobster) chemosensors cells react to stimuli based on thresholds of both stimulus concentration and duration and we can reasonably assume that this holds true across all decapod crustaceans. In relation to this study, it is not surprising then that tracking blue crabs do not show graded responses to changes in concentration when neuronal activity changes require order of magnitude changes in concentration.

Blue crabs modify their behavior in response to stimuli within approximately 0.2 s (200 ms) after the reception of an odorant spike (Figs. 5.9 and 5.10). In light of the cellular response patterns measured by Gomez and Atema (1996) it seems that, whatever the actual stimulus may be, crabs are receiving the odorant(s) in high enough doses to elicit cellular responses on the order of 50 ms or less in order to produce noticeable changes in behavior by 0.2 s. While the time scale of this behavior may seem rather rapid, other organisms also can detect, discriminate, and react to odorant stimuli within 0.25 s or less. Humans are able to detect an odorant within just one sniff and are able to modify the air flow through the nose within 160 ms of starting that sniff if an odorant is detected (Johnson *et al.* 2003). Rats that have been trained to discriminate between odorants respond with faster sniffing if a new odorant is detected and this discrimination and resultant increase in sniffing can be detected within 140 ms of sniff onset (Wesson *et al.* 2008). *Drosophila* tracking in odorant plumes are able to detect an odorant as they initially contact a chemical plume and reorient their flight trajectory to follow that plume within 250 ms of contact (Budick and Dickinson 2006). Moths can respond to odor within 300 ms (Mafra-Neto and Cardé 1994; Vickers and Baker 1994) and are able to discriminate between odorant sources that are separated by as little as 1 ms in time and 1

mm in space (Fadamiro *et al.* 1999). This is directly analogous to the period necessary for a blue crab to detect and then respond to a signal (Figure 5.8 and 5.9) and may indicate that blue crabs are processing data in much the same way as a tracking insect.

Despite these findings supporting behavioral reactions to stimuli on the order of milliseconds, previous measurements of tracking lobsters suggest that they are behaviorally responding to stimuli on the order of 2-4 s (Basil *et al.* 1995; Atema 1996). In this case, Basil *et al.* (1995) measured the turning behavior of lobsters and correlated these turns to bilateral signals (food odorants mixed with a dopamine tracer) detected in the area of the lobsters aesthetascs. While this behavior is occurring on an order of magnitude greater time scale than what was measured for along stream acceleration responses in the blue crabs, it is precisely the time scale over which blue crabs made turning decisions based on our threshold of a 5% COM shift (Figure 6.8). These data demonstrate that tracking blue crabs react to stimuli in the along-stream direction (antennules) on the order of milliseconds but take on the order of seconds to react to cross-stream information (legs), and that both these time scales are reasonably correlated with existing literature across a wide variety of organisms. The difference may reflect a number of neurological processes. Information mediating upstream motion simply may be processed faster because, in contrast to signals mediating turning, stimuli impinging on the antennules do not require spatial integration of multiple signals. Additionally, crabs may react to antennule data more quickly because along-stream movement is coupled with information from the mechanosensors, providing clear information about source directionality. Accordingly, crabs may react to cross-stream data more slowly because directionality of cross-stream fluctuations may be less "reliable" than along-

stream information at predicting the plume centerline. Regardless of the mechanism, these data suggest that lobsters may be reacting to stimuli in the along-stream direction on much shorter time scales than their reactions to cross-stream stimuli.

Crustacean chemosensor cells are able to discern changes in concentration over a stimulus threshold when, stimulus concentrations differ greatly (*i.e.,* > 10-fold) and stimulus integration times are long (Gomez and Atema 1996). As discussed, it is perhaps not surprising then that crabs do not respond to any concentration changes while tracking (Figs. 5.15-5.17). However, due to the increased concentration and spike contrast near the stimulus source and the observation that tracking blue crabs change their behavior close to the source, there is still a possibility that tracking blue crabs do respond in relation to changes in concentration or to changes in concentration combined with changes in another stimulus property, such as plume width. Due to the limitations of the 3DLIF system, the data from this dissertation do not extend all the way to the source (data was collected from 150 – 40 cm downstream of the source) and, in fact, stop short of where a shift to near source behavior would be expected. Further studies must be conducted to confirm that blue crabs do not utilize concentration shifts to mediate the final phases of tracking.

7.2.1.3 Signal intermittency

While the data do not support the hypothesis that tracking crabs do not respond to concentration with graded changes in behavior, the data do support the hypothesis that there is a positive interaction between intermittence and upstream tracking velocity. Even though the response to absolute concentration itself is binary (above or below threshold), crabs may respond to the frequency of spikes above threshold to produce a

functionally equivalent, graded behavior. Tracking in more homogenized plumes appears to be more difficult for crabs than tracking in more intermittent plumes. In particular, decreased intermittency associated with increased, bed induced plume homogenization causes a decrease in tracking performance (Figure 2.1) and success (Table 2.2) in tracking blue crabs. This is corroborated by the simultaneous data from this study indicating that crabs receiving more frequent spikes at their antennules (Continuous plume, Figure 5.1) had straighter paths (Figure 4.18) and faster along-stream velocities towards the source (Figure 4.13), resulting in shorter search times (Figure 4.4). In particular, as the time between spikes increases by ~50%, the mean search time increases roughly 30-50%, indicating a more or less direct relationship between the two variables. Tracking crabs were also more likely to stop (Figure 4.6) and spent a greater percent of their time stopped (Figure 4.5) in plumes where they had the least frequent contact with antennule concentration spikes. This suggests that crabs may employ "station keeping" as a viable method of regaining plume contact or assessing changes in plume properties. A tracking animal that has lost odorant plume contact may be able to maintain its position and, due to the filamentous nature of turbulent plumes, make contact with the odorant plume again when turbulent mixing directs a filament to that spot again (Cardé and Willis 2008). Additional information on the role of intermittence in modulating blue crab behavior was obtained as a part of this study by Dickman (2008), who was able to generally correlate the directedness of a blue crab's track with intermittency by overlaying crab tracks on fields of the intermittency factor from the Continuous plume (see Figure 5.9 in Dickman 2008). Dickman found that crabs made larger heading adjustments when the intermittency factor was less than roughly 0.02 and straightened

their paths towards the source when they were in regions with intermittency factors greater than 0.06.

Despite these generalized trends, the 3DLIF data also demonstrated that crabs are more likely to stop when they encounter more frequent antennule spikes (Figure 5.13). Frequency analysis demonstrated that post spike response is a function of spike frequency and that more frequent spikes produce the highest frequency of stops and decelerations in tracking crabs (Figure 5.14). However, more frequent spikes also are associated with accelerations, suggesting that spike frequency alone does not completely predict the subsequent response (*i.e.*, speeding up, slowing down, or stopping). There are two important conclusions from this data. The first is that while generally low frequencies result in halting upstream progress, the simple conclusion that all stopping events therefore are a response to stimulus absence, is false. Second, these data, taken together, indicate either a state dependency in a tracking crab's response to an odorant spike, or that spike frequency acts in conjunction with other signal parameters to determine the behavioral response. Crabs that encounter odorant spikes at a (relatively) lower frequency may stop in response to more frequent odorant spikes, which may generally be indicative of a crab approaching the source. Presumably, crabs that are already stopped would resume tracking in response to the reception of another concentration spike or a change in spike frequency. This would explain both the observation that both stopping and upstream movement are associated with higher spike frequencies, and the greater tendency for crabs in all more intermittent plumes to spend more time stopped. A related explanation for the variation in response to spike frequency may be that crabs change their threshold of what constitutes "more frequent spikes" as their spike encounter rate

changes with distance from the source (Figure 5.1). In this case, I would expect to see crabs stopping less frequently in response to more frequent spikes as they get closer to the source. Lastly, spike frequency, combined with other information, such as leg concentration data, may jointly mediate the response. Because signals at the legs have the potential to mediate the upstream progress of tracking crabs, signals arriving at the leg and antennules relatively concurrently may be more likely to induce upstream surges whereas antennule data in the absence of concentrated upstream leg signals may induce slowing or stopping. All of these hypotheses are testable with the current data set and I am continuing these evaluations.

Tracking moths tend to have a similar positive relationship between signal intermittency and continued motion towards the odorant source as displayed (in general) by blue crabs. Male *Cadra cautella* moths respond to turbulent filaments of pheromone by flying relatively due upwind compared to the cross-stream movement they exhibit when contacting an intact ribbon plume (Mafra-Neto and Cardé 1995). A similar upwind surge in response to pheromone filaments is seen in *Helicoverpa tea* (Quero *et al.* 2001), *Heliothis virescens* (Vickers and Baker 1996, 1997), and *Cadra cautella* (Mafra-Neto and Cardé 1995a, b, 1996). Cessation of upstream movement in response to signal homogenization has been demonstrated in the summerfruit tortrix moth (*Adoxophyes orana*; Kennedy *et al.* 1981), the oriental fruit moth (*Grapholita molesta*; Willis and Baker 1984), and the pink bollworm (*Pectinophora gossypiella*; Justus and Cardé 2002). Studies have also shown that the gypsy moth, *Lymantria dispar,* decreases air and ground speeds and reduces the frequency of their wingbeats while tracking in response to an increase in source pheromone concentration (10 ng to 1000 ng of pheromone; Charlton *et*

al. 1993). These moths additionally decreased the width of their flight tracks by steering at smaller angles (lower NGDR), indicating they were moving more directly upwind in straighter paths. Charlton *et al.* (1993) indicated that, while the turbulent dynamics of the odorant release or the environment did not change, the active width of the plume increased. Though this initially seems like a response directly dependent upon concentration, these effects may be a factor of an increased number of filaments above a fixed threshold, essentially creating a less intermittent and more homogenous plume. However, Justus and Cardé (2002) also demonstrated that not all moths rely on signal intermittency for successful odorant plume source location. When challenged with navigating a homogenous cloud of pheromone within a wind tunnel, the upstream progress of the almond moth, *Cadra cautella*, was not impeded by the constant olfactory signal (Justus and Cardé 2002) even though they react to single filaments by surging upstream (Mafra-Neto and Cardé 1995a,b, 1996). Similarly, *Drosophila mellanogaster* is another example of an insect that does not appear to rely on intermittent signals for successful odorant plume navigation as it can fly rapidly upwind while tracking a homogenized odorant plume (Budick and Dickinson 2006). Crayfish experience enhanced tracking performance when navigating a highly homogenous plume (Moore and Grills 1999), which appears to be unique among the decapod crustaceans, and spent more time stopped while tracking an odorant at pulse rates lower than 2 Hz (Kozlowski *et al.* 2003).

7.2.2 Complementarity and redundancy in sensory systems

Sensory systems are remarkable in that they each can serve multiple functions and interact in a complementary way, thereby providing a mechanism of redundancy that is

useful if one or more sensory systems is impaired (Simon and Levitt 2007). For instance, the visual and auditory systems of vertebrates can both help locate the source of a sound but the visual system has much better spatial resolution to determine the location of a visible sound source. In the absence of visual cues, the auditory system can still determine the direction of a sound but with less spatial precision. Conversely, the auditory system has better temporal resolution than the visual system, allowing greater acuity in auditory tasks such as speech recognition, yet the visual system can effectively supplement the auditory system under adverse listening conditions. For example, normally sighted individuals with hearing loss can determine the direction of a sound almost as well as normally sighted individuals without hearing loss (Simon and Levitt 2007). In the absence of visual cues, the weakly electric black ghost knifefish (*Apeteronotus albifrons*) utilizes tuberous electrosesnse, ampullary electrosense, and the lateral line canal system to "image" the location of prey (Nelson *et al.* 2002).

Determining functional redundancy versus complementarity is a complex task as organs within the same sense (*e.g.*, olfaction) can be functionally redundant for some tasks and function differentially for other tasks. For instance, Yamagishi *et al.* (2008) found that the odor-aversion learning ability of a slug, *Limax valentianus*, can be modulated through both the superior and the inferior tentacles, whereas Friedrich and Teyke (1998) demonstrated that the inferior tentacles are necessary for olfactory learning in the snail *Helix*, and the superior tentacles are necessary for the recall of olfactory memory. Chase and Croll (1981) also demonstrated that odor orientation to a distant (windborne) odor source by the snail *Achatina fulica*, is dependent upon the superior tentacles, while substrate trail following is dependent upon the inferior tentacles. There

is a functional division of tasks between different chemosensor populations in lobsters as well. Deafferentation experiments determined that chemoreceptors on different appendages of the lobster (*Homarus americanus*), specifically the antennules, leg segments and tips, and maxilipeds, mediate different functions while feeding, ranging from handling time (grasping, crushing) to initial identification of a viable food source (Derby and Atema 1982). In the spiny lobster (*Panulirus argus*), both the aesthetasc and non-aesthetasc pathways can mediate the search for food alone even though the two pathways project to different neuropils in the brain (Horner *et al.* 2004). Keller *et al.* (2003) performed an elegant deafferentation experiment and demonstrated that the antennule chemosensors of blue crabs play a significant role in mediating upstream motion while the leg chemosensors are important for orienting within the plume itself. That is, there is considerable specialization in the roles of each of these appendages, although either sensory population is sufficient for successful, if less efficient, search (Keller *et al.*, 2003).

Experiments in this dissertation support the findings of Keller *et al.* (2003) by specifically determining the signals available to each sensor population and the behaviors associated with signal changes at each population. Antennule chemosensors sample much higher in the water column than leg chemosensors, and are therefore in a region that experiences the greatest change in signal structure with elevated levels of turbulence caused by increasing bed roughness (Jackson *et al.* 2007). This decreased probability of encountering concentrated odorant signals at the height of the antennules with increased turbulence corresponds with a decrease in the speed (movement towards the source) of

tracking blue crabs (Figure 2.1b), indicating that the intermittency of concentrated odorant signals at the antennules is affecting the upstream motion of tracking blue crabs.

The leg chemosensors sample in a region of the plume that is the least affected by increased turbulence (Jackson *et al.* 2007) as the plume structure is more consistently homogenous due to the interaction with the bed. There was also very little evidence that the orientation of a blue crab was affected by increasing bed roughness (Figure 2.1a), supporting the role of the leg sensors in plume orientation by blue crabs. When the distance of the crab from the centerline of the plume was analyzed in respect to the proximity of a crab to the source, we found that the crabs tended to wander farther from the plume centerline at farther distances from the source and with increased bed roughness (Figure 2.3a). The width of the plume in these conditions is increased, thereby adding further weight to the argument that blue crabs use their leg chemosensors in turning. The results from the 3DLIF studies indicate that plume type had a significant effect on the NGDR of tracking crabs (Figure 4.18), with crabs that were in the Continuous plume traveling in more direct routes to the source than crabs in the other two plumes. Crabs also tended to increasingly straighten their paths as they approached the source, again corresponding to decreased plume width (Dickman 2008).

These results led me to consider the two processes of upstream motion and steering within a plume separately as they may not be equally affected by changes in plume structure by varying turbulence. Additionally, the experiments examining the effects of bed roughness on tracking behavior demonstrated that there were fundamental differences in the odorant signal properties in the regions of the boundary layer associated with each sensor population (Jackson *et al.* 2007). The antennules sample at a

height in the water column where the odorant field is characterized by high concentration bursts, large fluctuations, and a large intermittency factor compared to the region closer to the bed. The changes in these properties with increased bed roughness and the corresponding decreased speed of tracking blue crabs indicates that the antennules rely on the frequency of intense odorant bursts to mediate upstream motion. Chemosensors on the walking legs experience more homogenous plume structure and data indicates that they rely on contrast over the transverse spread of the plume to mediate cross-stream orientation. The 3DLIF studies provided the opportunity to test the relative contribution of each set of chemosensors to particular tracking behaviors and more precisely determine the properties of the specific signals that are important to each set of chemosensors. Analysis of the relative contribution of signals at the leg and antennule chemosensors on upstream motion revealed, surprisingly, that reception of signals at the leg chemosensors help to mediate upstream motion by reducing the reaction time to subsequent antennule stimulation (Figure 5.18). Interestingly, Aggio and de Freitas (2007) demonstrated that chemical stimuli (taurine) delivered to the dactyls, walking legs, or swimmerettes of *Callinectes danae* induced these crabs to move as if to grab something in front of them or begin walking, whereas the same stimuli delivered to the antennules did not evoke any movement. This supports the idea that, at least under certain circumstances, stimuli arriving at the leg chemosensors can help to initiate and perhaps sustain motion in tracking blue crabs. This new information highlights an important distinction that, while it is useful to consider the two processes of upstream motion and plume orientation separately, we must also remember that there can be a level

of sensory redundancy or complementarity between these sensor populations when we are interpreting reactions to stimuli.

7.2.3 The use of stimulus asymmetry

7.2.3.1 Cross-stream travel in relation to stimulus asymmetry

The chemical tracking ability of a variety of organisms is enhanced by input from spatially distributed sensors. Simultaneously comparing the input across multiple chemosensors is one way an organism can determine the most likely direction to travel in order to locate a stimulus (*i.e.*, in the direction of the sensor experiencing the highest concentration). *Drosophillia melanogaster* larvae utilize input from a pair of bilateral sensors to enhance their ability to navigate within an odorant gradient (chemotaxing) (Louis *et al.* 2008). Crayfish appear to compare information bilaterally from a pair of antennae to successfully track odorant plumes to their source (Kraus-Epley and Moore 2002; McMahon *et al.* 2005) as do lobsters (*Panulirus argus*: Reeder and Ache 1980; *Homarus americanus*: Devine and Atema 1982). Even humans utilize bilateral signals for source localization. While wearing individually appropriate hearing aides to counteract hearing loss, individuals that experience symmetric, bilateral hearing loss could determine the directionality of a noise more accurately than could individuals that experience hearing loss in only one ear (Simon 2005). While humans do not rely on their source of smell to locate a source, there is a measure of bilateralization to our odorant perception. In particular, when one nostril receives a stronger stimulus than the other nostril, the side receiving a stronger signal makes a disproportionately large contribution to our perception of the overall strength of the smell, termed the rule of partial summation (Cain 1977). Additionally, even though the olfactory epithelia from each nostril are not

connected in the peripheral areas, one nostril can learn to recognize a smell only presented to the other nostril, indicating the signals are shared in the brain (Mainland *et al.* 2002).

The antennules of the blue crab are too small and close together to provide a good spatial indication of its location within a chemical plume and hence, bilateral stimulus comparison has been suggested across the leg chemosensors as a mechanism by which tracking crabs can stay within a plume's transverse boundaries. In this scenario, crabs moving from side to side in the plume would compare the concentration of signals from the legs on one side of their body to signals from the legs on the other side of their body. For this strategy to work, leg sensors on one side of the crab's body would have to be substantially offset from the other set of legs in the cross-stream direction in order to create the potential for each set of legs to receive a different set of chemical signals.

Crabs preferentially reduce drag while tracking, if possible, by orienting their major body axis more closely parallel to the mean flow direction. In these cases, the leg sensors on either side of the crab are actually aligned with the mean flow vector and therefore bilateral (across-the-body) comparison may provide information regarding the along-stream concentration gradient, but will not sample the cross-stream gradient associated with plume edges. Despite this body orientation, tracking crabs are adept at staying within the plume boundaries, particularly while tracking the Continuous plume. We also see strong evidence that crabs tracking all plume types are able to make very sensitive cross-stream course corrections to more accurately approach the source location (Figure 6.1). Therefore, the specific input determining the contrast utilized by blue crabs

during cross-stream localization may not be constrained to comparisons across the major body axis.

The 3DLIF studies clearly indicate that blue crabs are able to detect and react to cross-stream directional concentration center of mass (COM) shifts by comparing signals across multiple leg sensors on the upstream side of their body. A shift in COM of less than 1 cm to the left or the right is enough to initiate turning behavior towards the direction of that concentration shift (Figure 6.8) and can be considered a form of edge detection. Chapter 2 demonstrated that the contrast between sensors of a fixed width increases as the sensors move farther to the side of the plume (Figure 2.3b). Therefore, crabs would find the sharpest contrast near the edge of a plume and shifts in the concentration COM across even a single set of leg sensors would be more dramatic at these locations if the crab began to exit the plume. This multi-point, gradient sensing strategy seems akin to the tracking behavior of the starfish, *Asterias forbesi*, which does not have a central nervous system and appears to compare chemical information across the tips of all its rays and moves in the direction of the ray with the highest concentration (Dale 1999). Despite the utility of such a design for bilaterally symmetric animals like crabs, which do not necessarily have to move in a fixed direction relative to their body axis, this is apparently the first demonstration of such a sensing scheme that does not strictly require bilateral contrast. It is unclear whether the underlying coding mechanisms are similar in bilateral animals capable of omni-directional movement vs. non-bilateral creatures such as echinoderms.

Crabs may use bilateral integration across their body as a secondary orientation strategy in particularly complex tracking situations. Tracking blue crabs are able to

follow the Meandering plume as it shifts from side to side but these crabs do so at an increased body angle with respect to the mean flow direction. As mentioned previously, this orientation increases their drag but will increase the concentration of the filaments reaching their antennule sensors. Adopting this position (~42˚) is also a direct balance between optimizing the potential for bilateral signal contrast across the body (90˚), versus optimizing the potential for identifying cross-stream signal shifts across a single set of leg sensors (0˚). Intermediate body angles in the case of the Meandering plume may be an optimal balance between being able to compare signal across a single set of upstream leg sensors while also being able to compare cross-stream signals bilaterally. This would be an advantageous strategy to give the crab the best possible chance for determining the source direction of a continually shifting odorant plume.

7.2.3.2 Downstream travel in relation to stimulus asymmetry

Tracking crabs did not always move forward or stop, as evinced by their occasional negative V_x (Figure 4.14). In particular, crabs in the Pulsed plume tended to spend the greatest percent of their track time traveling downstream, followed by crabs in the Meandering plume and then the Continuous plume (Figure 4.17). In other tracking organisms, downstream motion appears to be an attempt at regaining plume contact. When mature female *Brachymeria intermedia* wasps lost plume contact while tracking to host odors (gypsy moth pupae) or a conditioned odorant (vanilla), they displayed a variety of behaviors including flying in irregular patterns upwind and downwind while also making excursions in the vertical direction (Kerguelen and Cardé 1997). Tracking tsetse flies similarly make large course corrections when they lose contact with an

odorant, with the size of the course correction determining the degree to which the fly backtracked (Gibson and Brady 1985).

Backtracking serves to put a tracking organism in the last place that they detected an odorant, which increases their chances of requiring a signal lost due to either turbulent motion of the plume or by their own movement (*e.g.*, overshooting the source). Though this generally indicates that animals which lose contact with a plume should head downstream, blue crabs don't always turn downstream after long periods without an odorant signal. Crabs are much more likely to turn downstream in the Pulsed plume, which has great temporal variation. The physical distance between the odorant pulses as they are advected downstream indicates that, even along the plume centerline, crabs have a great possibility of being physically unable to make contact with the plume with any of their chemosensors at certain points during their track. Anecdotally, crabs in the Pulsed plume that did move backwards often were situated in a odorless packet of fluid between two odorant pulses and they were actively "chasing" the plume downstream (Page, *pers. obs.*). Such behavior is a bit unusual given the variety of evidence suggesting odorant perception initiates upstream movements in crabs and other creatures (*e.g.*, moths: Vickers 2000; freshwater eels: Carton and Montgomery 2003; nudibranchs: Wyeth *et al.* 2006; barnacle larvae: Pasternak *et al.* 2004).

One hypothesis for control of downstream movement is that crabs that lose contact with the plume at their upstream leg chemosensors and at their antennules may be induced to move downstream if they are still in contact with signal on their downstream legs. This provides a strongly asymmetric signal that may induce downstream locomotion, but is not liable to produce downstream movements under most conditions.

In general, odorant filaments arriving at the downstream leg sensors will be less concentrated than those arriving at the upstream leg sensors. Filament homogenization will occur partially due to turbulence acting over the additional advection time to reach the downstream legs and especially due to the interactions of the odorant filaments being homogenized as they pass around the body and through the upstream appendages of the crab. Images and physical measurements of dye flowing over a crab at different body angles to the flow confirm this significant plume homogenization due to its interaction with the upstream portions of the crab (Weissburg *et al.* 2003) and demonstrate that the plume is also much wider after this homogenization. The concentration of filaments available to the upstream chemosensors is clearly greater than the concentration of filaments available to downstream chemosensors, which, under Continuous plume conditions, would set up a clear, along-stream bilateral comparison for the crab. Unsurprisingly, we rarely observe downstream motion in the Continuous plume. Only under certain conditions of great spatial/temporal patchiness would stimulus asymmetry shift so that the downstream legs are receiving more concentrated stimuli than the upstream legs, and induce movements downstream. For instance, when a crab overshoots a source the upstream legs and antennules would no longer detect an odorant but the downstream legs could detect the odorant in a very high concentration. The relative contributions of flow and odorant information to a successful search was explored by Weissburg and Dusenbery (2002) when they compared the behavior real tracking crabs to computer simulations of tracking crabs that had their behavior altered based on various contributions of rheotactic and chemotactic search strategies. When chemotactic strategies had greater influence (low weightings according to their classification scheme)

simulated crabs were more likely to move downstream following blobs of simulated odorant. The data from this study suggest that the influence of chemotactic vs. rheotactic information can shift over the course of a single track and that chemotactic cues have the potential to outweigh rheotactic cues.

Due to the physical and biological limitations of this study, I did not collect concentration data on signals arriving at the downstream legs of tracking crabs. Additional analysis of the current 3D, simultaneous data set and additional experiments are necessary to test this and other hypotheses related to stimuli arriving at the downstream leg chemosensors.

7.2.4 Vertical integration

Initial measurements of animal height while tracking indicated that crabs are decreasing the height of their antennules as they approach the source (Figure 4.19). This decrease in antennule height appears to correspond to the decreasing dispersion height of the odorant plume, indicating that the crabs are employing some active mechanism for maintaining vertical contact with the plume. I hypothesize that blue crabs may actively scan in the vertical direction by raising and lowering their body if they lose stimulus contact in an attempt to re-establish contact. In that case, I expect to see some degree of "bobbing" (relatively rapid vertical body movement up-and-down) when crabs experience decreased frequency of odorant contact. This hypothesis is complicated by the earlier conclusion that signals arriving at the leg chemosensors help to mediate a crab's forward motion. This suggests that there may be similar integration mechanisms whereby signals (or loss of signals) at both the antennules and the legs mediate changes in the vertical direction. For instance, crabs that experience decreased stimulus frequency

at their antennules while retaining plume contact at the level of the leg sensors may continue upstream movement without bobbing and only initiate bobbing if both sensors experience an extended period of signal loss.

Maintaining contact with a plume in the vertical direction is not something that has been previously examined in blue crabs but has been addressed in the insect literature. Baker (1989) was the first to comment on the vertical behavior of moths tracking pheromone plumes. Baker qualitatively reported that there was a relationship between the vertical and horizontal movements during plume tracking by male *Heliothis virescens* but not during plume tracking by male *G. molesta*. Subsequently, Vickers and Baker (1996) quantitatively demonstrated that *H. virescens* moths maintained control over their vertical position within a plume while they were actively tracking; however, once they lost the plume, *H. virescens* made more vertical and lateral deviations in their flight path. A similar pattern of movement emerged in flight studies of tracking hawkmoths. Rutowski *et al.* (2009) demonstrated that male *M. sexta* moths vary their altitude while tracking a pheromone plume similarly to their horizontal deviations, thereby resulting in a series of loops of different radii that cut through the plume from all directions. They hypothesize that the mechanism by which moths control their vertical motion could be an expression of an active counter-turning search behavior extended to the vertical plane. Alternatively, they speculate that the behavior could be modulated by the frequency of encounters with odor filaments. Vertical deviations are also observed in tracking *Brachymeria intermedia* wasps when they lose contact with the plume (Kerguelen and Cardé 1997). Vertical control to remain in contact with the plume has also been addressed in the chambered nautilus. *Nautilus pompeii* orients within an

odorant plume in three dimensions and in particular seems to swim near the top or above the plume at larger distances from the source (> 100 cm) and switches to a position near the bottom or below the plume near the source (Basil *et al.*2000).

Vertical control while navigating a turbulent plume is a behavior that has been only been considered in swimming and flying organisms as it was believed that benthic organisms function only in two-dimensions. This sentiment is highlighted by Basil *et al.* (2000) who state that the chemically guided behavior of *Nautilus* is similar to that of lobsters and "animals that occupy similar benthic habitats…, except that *Nautilus* samples the vertical (or third) dimension as well." In light of the data from this dissertation, future studies examining the tracking behavior of benthic organisms should explicitly consider the organism's ability to orient in the vertical domain. Fortunately, the methods used in this dissertation allowed simultaneous measurement of vertical crab movements with the preceding antennule and leg stimuli records, which is the type of data necessary to answer the novel question of what stimulus properties are controlling vertical movement in tracking blue crabs. An analysis of this behavior is ongoing and I hope to more thoroughly address these vertical control mechanisms in a later publication.

7.2.5 Competing demands during signal acquisition

While foraging, blue crabs must balance conflicting demands from the hydrodynamic environment (drag) and their need to assess available chemical signals (Weissburg *et al.* 2003). Weissburg *et al.* (2003) found that in the absence of chemical signal at high and low flows (10 and 5 cm s^{-1}, respectively), crabs preferentially align their major body axis more closely with the mean flow direction (~13-18˚, according to the angle conventions of this thesis), thereby decreasing drag and correspondingly

decreasing the energy required for upstream motion. Crabs tracking an odorant in a high velocity flow oriented their body axis similarly (~18˚), again reducing drag while tracking. However, crabs tracking an odorant in the low velocity flow oriented their body at steeper angles to the flow (~38˚), thereby incurring greater drag costs, but increasing the concentration of odorant signals impinging on their chemosensors.

The experiments performed for this dissertation examined the body angle orientation of blue crabs under constant mean velocity conditions (5 cm s^{-1}, corresponding to the low velocity of Weissburg *et al.* [2003]) but with variation of the odorant plume structure. Successful trackers in the Control and Pulsed plumes preferentially oriented their body at low angles (18 and 22˚, respectively; Figure 6.9), more closely mimicking the body angles of crabs tracking in high velocities from the Weissburg *et al.* (2003) study. This implies that these searchers were receiving signals of high enough concentration at their chemosensors that they could afford to reduce the drag on their body by aligning their major axis more closely with the mean flow direction and still track successfully. Conversely, crabs in the Meandering plume oriented their body at much steeper angles (~42˚) suggesting that the signal that they received was weak enough that they had to sacrifice drag to increase odorant concentration at their chemosensors. This is corroborated by the plume measurements indicating that the mean concentration of the Continuous plume was the greatest, followed by the Pulsed and then Meandering plume, which was the most diffuse (Dickman 2008). In this case, the crabs are making a tradeoff in body angle based on the difficulty of the task as opposed to being based primarily on the drag cost as seen in Weissburg *et al.* (2003). We also see a small but significant decrease in body angle of crabs in all plumes in the 1 s after they

receive a spike at their antennules (Figure 6.9), in keeping with the idea that increased signal concentration is balanced by decreasing drag costs. These crabs significantly increase their body angle to more directly face the flow if they have not received a spike within the previous 1 s.

This problem of balancing energy efficiency with signal acquisition has not been well explored. Bees display tradeoffs between speed and accuracy when visually attempting to locate a food source (Chittka and Spaethe 2007) and moths may perform similar energetic tradeoffs between drag and signal acquisition while tracking in progressively stronger flows (Zanen and Cardé 1999). Most recently, MacIver *et al.* (2009) describe an elegant sensory/energy tradeoff related to drag in the knifefish, *Apteronotus albifrons*. *A. albifrons* is weakly electric and uses this sensory ability to detect prey within a cylindrical volume around its body. To maximize the volume being scanned for prey, *A. albifrons* can change the pitch at which it swims so that the back portion of the fish is scanning fluid that has not already been scanned by the front of the fish (as is the case in straight swimming). However, changing the body pitch angle from 0˚ to 30˚ (the observed pitch angle during prey search behavior) results in a doubling of body drag in exchange for a 30% higher volume scan rate. This seems directly analogous to the behavioral tradeoffs that we see in the blue crabs whereby some degree of energetic cost is deemed acceptable based on the sensory advantage that it confers. Hopefully future research into the knifefish postures during prey search will observe body pitch under a variety of environmental conditions, allowing more direct comparisons to be made between the two systems. From what we know about the tracking postures of blue crabs, I would expect knifefish to adopt increasingly smaller

angles as they are faced with prey search in faster flows where drag would be increased. However, electrosensory perception itself should not be significantly affected by turbulence and therefore I would not expect to see the body pitch change in relation to environments with more environmental turbulence across the same velocity, as examined in the bed roughness experiments (Chapter 2).

7.2.6 State dependent reactions

7.2.6.1 State dependent behaviors associated with tracking

The chemosensitivity of an organism, and therefore its behavior, can be directly affected by various internal and external cues. Though some cues elicit the same behavioral response regardless of when and under what conditions they are received, other cues can elicit very different behavioral responses dependent upon the internal and external state of the organism at the time of cue reception. These state-dependencies are an important version of behavioral plasticity that can directly contribute to an organism's fitness. For instance, nutritional state can affect the web building investment of the western black widow spider, *Latrodectus hesperus,* with well-fed spiders producing more silk and building denser webs than poorly fed spiders (Salomon 2006). Because web construction is energetically costly but web persistence is high, spiders respond with a building investment that is based on nutritional state. In some cases, these state dependencies affecting tracking behavior are based on these sorts of relatively long term processes such as hormonal changes, activity state, or hunger. For instance, male gypsy moths are more sensitive to pheromones at the beginning of scotophase (dark period) than they are during photophase (light period) even though the level of random locomotory activity is otherwise depressed during this time period (Linn *et al.* 1992).

Zimmer-Faust *et al.* (1996) clearly demonstrated that the locomotory state of an organism could also affect its sensitivity to chemical stimuli. Blue crabs and spiny lobsters both responded significantly to prey odor (bivalve exudates) only during phases where they were active, rather than resting, and the concentration of chemical stimuli (ATP) needed to evoke a behavioral response in resting lobsters was over two orders of magnitude higher than in active lobsters. As mentioned, Moir and Weissburg (2008) clearly demonstrated that hunger level and size affect the response of crabs to odorants and similarly the metabolic state (hunger level) also affects the response level of *Drosophila mellanogaster* to particular odorants (Quinn *et al.* 1974; Ruebenbauer *et al.* 2008). Different species within the same genus and under the "same" state may also demonstrate drastically different responses to that state. Walking *Drosophila mellanogaster*, which have been infected by the endosymbiotic bacteria Wolbachia, experience decreased odor localization ability while infected individuals of *Drosophila simulans* experience increased odorant localization ability (Peng *et al.* 2008).

Other state dependent processes operate on a much shorter time scale with more immediate benefit. Spinothalamic tract (STT) neurons respond to histamines and transmit signals to the brain that results in an itch, but STT neurons also exhibit spontaneous activity and activity due to painful stimuli. While an animal would receive a benefit from scratching an itch, they would likely cause more irritation if they scratched an area reacting to a painful stimuli. Davidson *et al.* (2009) demonstrated that STT neuron activity in a state induced by histamines can be reduced by scratching, thereby inhibiting the transmission of an itch to the brain. Conversely, when the neurons are evoked with a painful stimulus (capsaicin), scratching does not inhibit STT activity.

There are also plenty of state-dependent reactions to odorant stimuli. The descending interneurons of a male silkworm moths, *Bombyx mori*, exhibit a state dependent response to pheromone reception, which trigger a particular suite of behaviors (Mishima and Kanzaki 1998). This 'zigzag' walking is characterized by wing vibrations, abdominal ruddering (twisting of the thorax), and a distinctive pattern of head-turning movements (Kanzaki *et al.* 1994). Head turning is directly triggered by pheromonal stimulation of the descending interneurons (Mishima and Kanzaki 1998), causing their distinctive flipflopping neural activity between a high and a low firing frequency (Olberg 1983). The flipflop switch causes the moth head to swing to the opposite direction with each flip so continual frequency changes cause the head to swing constantly to the left and right. The body of the moth follows the direction of the head while the moth is tracking, thereby causing a zigzag type pattern. Though male *B. mori* moths cannot fly, Mishima and Kanzaki (1998) propose that the motor patterns of the zigzag walking behavior are similar enough to flying moth species (*e.g., Manduca sexta;* Kanzaki 1998) that flying may be induced by similar neural mechanisms.

The experiments performed in this dissertation revealed several incidences of what may be state dependent behavior in tracking blue crabs in response to specific chemical signals. The upstream velocity of an animal prior to receiving an antennule spike had a direct bearing on its reaction to that antennule spike. Crabs that were traveling at above average velocity immediately prior to receiving a spike at their antennules generally continued to travel at above-average velocity after receiving that spike (Figure 5.8), whereas crabs previously traveling at below-average velocity took longer to reach above average velocity after receiving an antennule spike (Figure 5.9).

Similarly, whether a crab was accelerating or decelerating prior to receiving an antennule spike determined their reaction to that spike; crabs that were decelerating took longer to accelerate than animals that were previously accelerating (Figure 5.11). Earlier data demonstrate that crabs are capable of responding rapidly to stimuli. In particular, crabs traveling at below average velocity are able to accelerate to above average velocity within 0.25 s of receiving an antennule concentration spike (Figure 5.9). Additionally, crabs decelerating prior to receiving an antennule spike are capable of accelerating within 0.25 s of receiving an antennule spike (Figure 5.10). These data suggest that the post spike acceleration patterns (Figs. 5.10 and 5.11) of blue crabs are an effect of signal processing and not simply a time lag due to a motor effect. The frequency of spike reception also seems to cause a state dependent reaction by blue crabs, as more frequent spike reception is associated with slowing down and stopping (Figure 5.13) but the raw interspike interval data does not directly predict the velocity patterns of a tracking crab (Figure 5.14).

There also appeared to be cross-stream state dependent reactions to stimuli at the legs. Crabs in the Continuous plume take the least amount of time to react to a concentration COM shift at their upstream legs compared to crabs in the Meandering and Pulsed plumes (Figure 6.8). This shift in response time may be in relation to the frequency or perceived reliability of those COM shifts. More frequent and more concentrated signals may also mediate the body angle of the crab, as crabs in the Continuous and Pulsed plumes track at shallower body angles to the flow than do crabs in the Meandering plume (Figure6.9).

7.2.6.2 State dependent behaviors not associated with tracking

During the studies in this dissertation, potential state dependent reactions were also observed while crabs were not tracking. I observed that crabs that did not leave the test cage within the designated period of 10 minutes showed vigorous activity when exposed to the odorant by moving mouthparts and rapidly flicking and scraping antennae with their legs as if they were cleaning themselves. Despite the apparent aversion to a food related cue, these non-tracking animals readily accepted and consumed pieces of shrimp (the source of the stimulus) after being removed from the flume. This behavior may be resolved in light of other research conducted on sniffing in aquatic and terrestrial systems.

Prior studies have demonstrated that flicking antennae is how new odorant samples are introduced to the aesthetascs (Koehl 2006). This behavior is also observed in animals without aesthetascs. The tentacles of snails (*Helix aspersa*) repeatedly twitch and quiver while tracking an odorant and these behaviors are thought to increase access to odorant molecules by decreasing the boundary layer at the tentacle and to remove entrained odorant from the surface of the tentacle (Lemaire and Chase 1998). The rapid sniffing behavior of rats in response to a positive stimuli has also been demonstrated as a way to acquire a stimulus more rapidly once it is available (Wesson *et al.* 2009). Rapid sniffing is also observed without any odorant present but when there is anticipation of an odorant (Kepecs *et al.* 2007), or when a rat has been preconditioned to an odorant and a novel odorant is presented (Wesson *et al.* 2008), thereby increasing the sampling rate in the hope of detecting the desired stimulus.

It seems clear from the behavior of the non-tracking crabs that they were intent on removing the food related odorant from their chemosensors and resampling at a high rate, akin to the sniffing behavior seen in rats. In light of the findings of Wesson *et al.* (2008) and Kepecs *et al.* (2007), it may be that crabs were clearing their chemosensors in anticipation of receiving an immediately more important stimulus than food, such as pheromones. Blue crabs were housed in a set of recirculating seawater tanks and no attempt was made to separate males from females. Consequently, I would occasionally find male blue crabs guarding females, crabs actively copulating, or males displaying courtship stationary paddling (Kamio *et al.* 2008), which serves to direct pheromones at a female receiver. Crabs actively engaged in any of these behaviors were not selected for trials that day, assuming interest in mating or actively searching for a mate would outweigh any interest in actively tracking food. However, crabs not engaged in any of these behaviors may still have been more interested in mating than in finding food, especially if other crabs are releasing pheromones, thereby elevating background pheromone concentrations in all the recirculating tanks. Elevated background levels of pheromone would also cause crabs to experience an abrupt loss of pheromone contact when they are place in the flume, which utilized a separate source of recirculating seawater than the holding tanks.

The hypothesis that non tracking, yet highly active crabs were more interested in finding a mate than finding food is not discernable with the data collected from this thesis as the status of other crabs in the tanks was not recorded and no digital records were kept on crabs that did not exit the cage. However, a preference for pheromone odorant over food odorant has been demonstrated in the crayfish (*Orconectes virilis*) and that

preference was not altered by the hunger level of the crayfish (Pecor and Hazlett 2008). Crabs were fed small amounts of shrimp roughly every other day while they were kept at Georgia Tech. Consequently, crabs were generally hungry but had not been starved, which may affect their responsiveness to a food odorant in the presence of a pheromone or the search for a mate. Male guppies (*Poecilia reticulata*) choose food over a mate when presented with stimuli from each of those resources (Abrahams 1993) and hermit crabs (*Clibanarius vittatus*) were less likely to form mating pairs and spent less time as a mating pair when food stimuli were present (Hazlett and Rittschof 2005). Crabs may have been more likely to forage, regardless of reproductive state if they had been under more drastic conditions of starvation. These types of tradeoffs are ecologically important from several standpoints. Dwindling availability of food causes some species to avoid mating and preferentially forage first, which evolutionarily could be a mechanism to make sure that they have the resources to meet the demands of courting, or to avoid introducing more individuals into an environment that is already resource depleted. Alternatively, prey may find a temporal refuge from predators if their predators preferentially choose to find a mate over finding food. However, as demonstrated by the blue crabs in this study, just because a predator is not interested in expending energy to search for food over finding a mate doesn't mean it will not take food if the opportunity presents itself.

7.3 Broader implications

The work from this dissertation has helped to develop and utilized a novel, 3D, measurement system to simultaneously measure the physical properties of odorant stimuli arriving at an actively tracking blue crab's chemosensors and the resulting navigational

behavior (Chapter 3). Measurements from this system (Chapters 4-6) and from the bed roughness observations (Chapter 2) have broader implications for the development of better tracking algorithms for autonomous robots, understanding how chemosensory signals are processed in the nervous system, and how chemosensory mediated processes can have large scale, ecological effects.

7.3.1 Autonomous vehicles – suggestions for search algorithms

Researchers working to develop autonomous robots that can track turbulent chemical plumes have taken inspiration from biological sources, trying to adopt the salient features that seem to be governing the search behavior of insects and crustaceans, in particular. The more recent attempts have focused on combining information from flow with concentration information to steer the robots within a chemical plume and have been based on the upstream surges and cross-stream steering of moths and lobsters, respectively (Hayes *et al.* 2002; Grasso and Atema 2002). Farrell *et al.* (2005) implemented a 'behavior based planning' strategy whereby individual behaviors, such as reacquiring a lost signal, are based on strategies in insects, and synthesized from the bottom up to create a comprehensive tracking strategy. Utilizing this approach, they were able to construct a long-range autonomous underwater vehicle that could track a plume over hundreds of meters but was only accurate within 13 meters.

Despite these attempts, applying search strategies derived from interpretation of animal navigation behavior has not allowed autonomous agents to replicate the level of success of the animal itself. The main problem facing the engineers of these autonomous vehicles is that there is no clear consensus on the details of plume structure and dynamics which are useful, every piece of information that can be derived from these plumes can

potentially be used to generate a plume-tracking search algorithm (Naeem *et al.*2007). By simultaneously measuring odorant properties at a tracking blue crab's chemosensors and the subsequent behavioral decisions, this dissertation has been able to correlate these two variables more closely than has ever been accomplished before. The information from these studies clarifies the signal properties that are important for blue crabs to track turbulent chemical plumes and this information can then be translated into a variety of algorithms for autonomous search.

Plume finding, plume maintaining, plume reacquiring, and source declaration are considered the primary subtasks that compose chemosensory related search (Farrell *et al.* 2004). The data from this dissertation directly contribute to maintaining and reacquiring plume contact and suggest a wide range of strategies not currently being explored by the insect and lobster based autonomous vehicles. Insect-based algorithms for maintaining plume contact involve moving directly upstream as soon as the vehicle detects an odor concentration greater than a predefined threshold. If the robot does not detect another above threshold concentration within a prescribed time, it switches to a cross-stream (left-right) counter turning behavior (Naeem *et al.* 2007). To reacquire the plume after the searcher loses contact for an even greater time threshold, autonomous vehicles based on insect strategies switch into a dramatic counter-turning pattern that moves the vehicle in all (2D) directions relative to the last detection point, rather than just cross-stream (Farrell *et al.* 2003). Lobster based plume navigation strategies depend on two spatially separated sensors mimicking the chemosensory input from each of a lobster's antennules while tracking. This strategy is very similar to insects as vehicles are programmed to move upstream if signals from the two sensors indicate that the vehicle is within the

plume (Grasso and Atema 2002). However, upon losing the plume the vehicle casts to the side corresponding to the sensor that last detected a strong concentration rather than randomly moving from side to side.

Incorporation of these basic underpinnings of insect and lobster tracking behavior has advanced the abilities of autonomous plume tracking vehicles to track over longer distances than ever before and determine the plume source more accurately. However, as mentioned by Naeem *et al.* (2007), the spatial and temporal characteristics of a turbulent chemical plume are complex, and precisely defining which aspects of that structure are salient for successful plume navigation is an important task. Our new understanding of what properties are important to blue crabs while tracking and what the behavioral responses are to those properties provides a framework to develop a new generation of even more successful plume tracking vehicles. Specifically, looking at the effects of thresholds on defining signals or the absence of signals, the specific reactions to spatial and temporal intermittency, how state dependencies affect behavior, and the importance of vertical integration of signals all provide new information about how to create a more successful autonomous tracker.

Currently, the plume tracking strategies of autonomous vehicles rely on thresholds that are fixed (Sandini *et al.* 1993; Belanger and Willis 1998; Farrell *et al.* 2003). Blue crabs do have a base detection threshold for chemosensory stimuli (pulse concentration and duration; Gomez and Atema 1996) but crabs tracking a chemical plume appear to use a threshold relative to the concentrations they are experiencing on a per plume basis (Figure 4.3) rather than an absolute threshold based detection. This ensures a substantial amount of contrast between the background odorant levels and what

odorant spikes are considered behaviorally relevant by a tracking crab. Autonomous vehicles should employ a similarly flexible sensing system that adjusts the measure of a salient plume feature based on the average concentration impinging on the sensor.

Spatial intermittency was introduced in the 3DLIF experiments by causing the plume to meander. The crab's ability to follow this meander in the cross-stream direction was critically dependent upon its ability to constantly evaluate the transverse concentration shifts in the plume. Lobster based search strategies compare signal across two sensors in a binary manner, determining which sensor is experiencing the greatest concentration and shifting in that direction. This type of strategy was a very poor indicator of the direction that a crab would turn while tracking. Instead, crabs appeared to compare information across multiple sensors to determine the concentration center of mass at a given time point and then determine the cross-stream shifts of that center of mass with time. Crabs would turn in the direction of the cross-stream center of mass shift when it shifted roughly 1 cm to either side over a single time step (~0.2 s). This is another case of a threshold being relative rather than absolute and suggests that comparing signals across an array of sensors over time will be a more reliable method of maintaining plume contact than a binary evaluation of which side of the robot senses the highest concentration.

Increased temporal intermittency (introduced by the Pulsed plume) is correlated to longer stop times in tracking crabs (Figure 4.7), implying that crabs may employ a "station keeping" strategy (Cardé and Willis 2008) to regain plume contact once they have lost it. Search algorithms for autonomous vehicles tend to employ some form of a cross-stream search behavior when plume contact has been lost for a certain amount of

time, rather than causing the vehicle to stop forward motion. Earlier vehicles were programmed to move backwards to try to regain signal downstream. All three of these strategies (staying still, moving cross stream, and moving downstream) seem to be employed at some point along a crab's track but the data suggest that crabs are making the decision about which strategy to used based on additional information about the plume. For instance, crabs in the Pulsed plume that did move backwards often were situated in a odorless packet of fluid between two odorant pulses and they were actively "chasing" the plume downstream (Page, *pers. obs.*). Crabs that lose contact with the plume at their upstream leg chemosensors and at their antennules may be induced to move downstream if they are still in contact with signal on their downstream legs. This provides a strongly asymmetric signal that may induce downstream locomotion, but is not liable to produce downstream movements under most conditions. Only under certain conditions of great spatial/temporal patchiness would stimulus asymmetry shift so that the downstream legs are receiving more concentrated stimuli than the upstream legs, and induce movements downstream. Autonomous vehicles should take advantage of this specific stimulus asymmetry by employing chemical sensors on the downstream end of the vehicle. A state dependent preference should be given to the information at the upstream chemosensors when they are receiving adequate signal to continue upstream movement, but preference should be given to signals arriving at the downstream chemosensors when the other sets of sensors are without signal. This would ensure that vehicles only move downstream when significant stimulus asymmetry indicates that they are exiting the plume by moving upstream of it. For tracking crabs, this shift may indicate that they are about ready to overshoot the source of the plume and therefore

these downstream sensors may provide information critical to increase the accuracy of source localization. This strategy also implies that there should be a state dependent tradeoff between the importance of mechanosensory vs. chemosensory cues to the behavior of the robot.

The tracking behavior of blue crabs appears highly state dependent and state dependency has been integrated into the current tracking algorithms employed by autonomous vehicles. For example, a search algorithm may rely on the time between filament contacts to shift the vehicle through states designated as plume finding, maintaining, regaining, or source finding. However, in the same algorithm, filament contact causes the vehicle to move upstream independent of the events preceding that contact and therefore independent of the state of the vehicle. Blue crabs actually show a state dependent response to filament contact based on the previous rate of filament interception, and in particular, more frequent contacts generally cause crabs to slow upstream motion or stop moving altogether (Figure 5.13). This is in complete contrast to a search algorithm that would initiate repeated bursts of upstream movement in response to more frequent contact of concentration spikes. However, reversing the search algorithm of autonomous vehicles to decelerate or stop in response to more frequent spikes would be unrealistic as well. Crabs also accelerate in response to receiving more frequent concentration spikes (Figure 5.14), though not as frequently as they decelerate or stop. This indicates that crabs are assessing the frequency of spike contact in conjunction with another signal to determine their reaction to receiving a concentration spike. Though we do not have a full understanding of these multiple signals as of yet, search algorithms should have a mechanism for determining spike frequency/changes in spike

frequency and reacting to those changes rather than just reacting to spike reception as a single event.

Current chemosensory search algorithms have focused on the two dimensional (along-stream and cross-stream) problem of finding an odor plume source. Data suggest that crabs are additionally utilizing information in the third dimension (depth) to guide their searches. During these vertical shifts, crabs are placing their antennules in regions of relatively consistent intermittency, thereby causing them to decrease their height as they get closer to the source and the plume is not as spread out. Autonomous searchers would likely benefit from having a chemosensor that can change height to vertically follow and provide information about specific plume properties or having a vertical array of chemosensors that can constantly monitor the plume at multiple heights. Utilizing this third-dimension provides another signal for the vehicle to help keep it within the plume boundaries. Additionally, concentration compared across a vertical sensor array or compared as a single sensor changes height will help determine the upper limit of the plume and provide information as to what height provides the most useful signal for the vehicle while tracking (*e.g.,* intermediate level of intermittency).

7.3.2 Nervous system organization – processing chemosensory cues

This research has clearly demonstrated the importance of signal intermittency to the successful navigation of a turbulent plume by a blue crab. In particular, signal intermittency appears to instigate state dependent shifts in behavioral patterns, particularly around intermittency factors of 0.02 and 0.06 (Dickman 2008). These data indicate that an intermittent threshold may be causing a fundamental change in the excitement patterns within the nervous system that alters the behavioral output associated

with the input of subsequent stimuli, and provides a testable intermittency level with which to search for this neurological activity.

While it is clear that crabs use information from multiple pathways to mediate successful tracking, we have less information about how multiple sources are integrated to produce behavior. This study indicates that state dependent shifts are responsible for the relative contribution of mechanosensory and chemosensory inputs towards producing behavior. Evidence from multi-modal sensory integration in vertebrates suggests that when the input of two types of stimuli are processed in the brain, the stimulus that is more discontinuous (and therefore considered more salient) becomes the modulating stimuli (Shimojo and Shams 2001). This seems to be the case in tracking crabs: mechanosensory signals would remain more or less constant over the entirety of the track and the chemosensory signals would be the most discontinuous across all sensors. This concept also would explain the downstream reversal seen in some tracking crabs if they indeed are reversing in response to loss of odorant at all sensors except for the downstream legs. This sort of extreme discontinuity in the chemical signal would cause the chemosensory information to overwhelm the mechanosensory information in guiding the crab's behavior.

Data also indicate that crabs behaviorally respond to information arriving at the two populations of chemosensors (antennule and legs) on different time scales. Reactions to changing stimuli across the leg chemosensors may be inhibitory to cross-stream movement until a directional shift is sustained for a certain period of time. Alternatively, the amount of time to process the more complicated and less reliable cross-stream concentration shifts may be greater than the processing time for signals arriving at

the antennules. Longer processing time for integrating more pieces of information to produce a response has been well documented in the speech patterns of humans. Peters *et al*. (1989) demonstrated that the acoustical onset time of voicing after a stimuli increased as adult speakers were required to respond with one syllable words, polysyllabic words, and full sentences. Additionally, immediate responses required more processing time than delayed responses. It seems then that the longer reaction time to a cross-stream stimulus may simply be a factor of integrating stimuli across the chemosensory cells on all the walking legs (upstream and downstream) and the signals from the ends of the claws and swimmerettes to produce a reliable behavior.

7.3.3 Ecological interactions

The data from this dissertation highlight the complex interactions between turbulent properties of odorant plumes and the sensory capabilities of blue crabs as they navigate to a source. Blue crabs do utilize levels of high contrast to mediate their along-stream movement, but they employ a flexible threshold that is related to the concentration of the filaments they are experiencing in a particular plume. The large-scale increase of both spatial and temporal intermittency negatively affect the tracking efficiency of blue crabs but crabs have a greater difficulty tracking a Meandering plume than a Pulsed plume. In the Meandering plume, high values of intermittency occur at greater distances from the centerline due to cross-stream mixing. Thus, intermittency may be a poor indicator of coincidence with the plume center under these conditions. Average concentration in the Meandering plume is also reduced by an order of magnitude compared to the Continuous plume. These data suggest that a combination of intermittency and plume concentration may be an indicator of the level of difficulty that a

plume presents to a tracking crab. However, in the Pulsed plume crabs only experience decreased average concentrations and they have an intermediate search time compared to the two other plumes.

From the perspective of a prey species such as a pumping *Mercenaria mercenaria*, being able to homogenize their chemical exudates through behavioral or environmental means is not as effective camouflage as being able to induce significant meander to their chemical exudates, such that the intermittency will not be a reliable indicator of the plume centerline. Settling downstream of structural roughness elements in estuaries, such as oyster clumps or sea grass stands, will induce additional mixing that could cause a plume to meander. Areas of high flow velocity will also confer an advantage to prey primarily because crabs will reduce their angles to the flow while tracking to reduce drag, thereby decreasing the area over which they can perceive contrast and increasing the likelihood of cross-stream error while tracking.

Blue crabs are high-spatial, low-temporal signal integrators, which allow us to compare the responses of blue crabs to stimuli while tracking to other tracking organisms based on their particular spatial and temporal integration abilities. Blue crabs appear to rely on signal intermittence as an indicator of position within a chemical plume as they track plumes too quickly to derive location within the plume based on gradients of odorant concentration. This strategy is likely to be used by other organisms that rapidly track chemical plumes, such as sharks or eels, and in particular other decapod crustaceans such as lobsters or crayfish. The data reveal that blue crabs are integrating information over multiple chemosensors and their cross-stream direction of travel is based on determining the directional shift in concentration across multiple sensors. This appears in

contrast to starfish and lobsters, which turn towards the sensor experiencing the high concentration.

7.4 Unique contributions and applications

This dissertation resulted in a wealth of spatially and temporally dense data on the tracking behavior of blue crabs. Though analysis is on-going, the information already derived from these data substantially increased our understanding of the mechanisms of successful olfactory search. Novel contributions include:

- Crabs broadly respond to stimulus concentration changes with a simple step function, whereby concentrations over a certain threshold elicit a behavioral response.
- This step function is augmented by state changes, which alter reactions to subsequent stimuli.
- Crabs respond to concentration spikes that are on average two orders of magnitude greater than the mean concentration of the plume.
- Crabs modify their along-stream response to stimuli within approximately 0.2 s but modify their cross-stream response to stimuli on the order of 2-4 s.
- Cross-stream course correction is mediated by directional shifts in the cross-stream concentration center of mass, which can be detected bilaterally across both sets of leg sensors, or across a single set of leg sensors.
- Crabs respond to the intermittency of antennule concentration stimuli based on state; however, increased frequency of stimuli at the antennules is most commonly associated with decelerating or stopping, while decreased frequency of stimuli is most commonly associated with accelerating.

- Antennule and leg chemosensors are primarily associated with different aspects of plume orientation (along-stream and cross-stream motion, respectively), but rather than being purely complementary, they have some level of functional redundancy as signals at the leg chemosensors can help modulate along-stream motion.
- Blue crabs vertically integrate signals at their antennules by raising and lowering their carapace while tracking; specifically, blue crabs lower their body as they approach the source in apparent concordance with the decreasing dispersion height of the odorant plume.
- Blue crabs preferentially rotate their body in the direction they are walking yet cross-stream turns themselves are largely translational.
- Odorant reception at the antennules generally produces body rotation in tracking crabs and the particular rotational response appears to balance the hydrodynamic and chemosensory demands in a state-dependent manner.

These characteristics of the tracking behavior of blue crabs have elucidated a wide range of navigational strategies that have yet to be exploited by autonomous vehicles. Suggestions for improving autonomous search algorithms include:

- Thresholds relative to average perceived concentration rather than an absolute threshold.
- Multiple sensors in the cross-stream direction to mediate course correction by detecting shifts in the concentration center of mass.
- Stopping in response to total loss of plume contact across all sensors.

- Downstream sensors that initiate downstream movement when sensing the plume in the absence of concurrent plume sensing at any other sensors.
- Slowing or stopping movement in response to more frequent stimulus contact.
- Vertical sensing arrays (or a sensor with the ability to move vertically in response to changing plume conditions) to determine the upper boundary of the plume and the spatial distribution of the plume vertically.

This study highlighted the benefits of using the blue crab as a model organism for studying odor mediated navigation strategies. Features of the blue crab that are particularly useful as a model organism include:

- Three-dimensional array of sensors that display varying degrees of functional complementarity and redundancy.
- Clear ability to interpret and respond to signal intermittency rapidly (*i.e.*, < 0.25 s).
- Threshold mediated response to signal concentration based on present signal-to-noise conditions.
- State-dependent responses in a variety of behaviors.
- Body rotation balancing input from hydrodynamical and chemosensory cues.
- A sensor span that is related to the integral length scale of the plume.
- Three-dimensional orientation (*i.e.*, along-stream, cross-stream, vertical) while tracking.

7.5 Future directions

The 3DLIF method for collecting simultaneous plume concentration and crab behavior data resulted in a extraordinarily rich and rewarding data set. Due to the volume and detail of these data, it was beyond the scope of this dissertation to exhaustively analyze all aspects of the data and, consequently, there are many directions for future study within the current data set to gain an even more comprehensive understanding of the mechanisms of odor mediated navigation. Inevitably, a greater understanding of these data will generate more questions than answers, but there are several lines of analysis that I plan to pursue to complement the analyses presented in this dissertation. There are also many questions that cannot be answered by analysis of the current data set. These questions suggest areas for future study that are natural extensions of the experiments presented in this dissertation. The following are some suggestions for future study, both analyses of the current data set and suggestions for future experiments.

- Data were only collected from ~150–40 cm downstream of the source. Three-dimensional, simultaneous measurements of plume properties and crab tracking behavior need to be made all the way to the source location to allow better understanding of near source search behavior.
- Similarly, leg concentration data were only collected for the upstream set of leg sensors due to particular aspects of crab tracking behavior and consequent limitations of the 3DLIF system to measure concentration at the downstream leg chemosensors. Measuring the simultaneous signal properties impinging on these downstream chemosensors is important for testing hypotheses regarding the mechanisms driving downstream movement in a tracking crab.

- Analysis of the current data set may elucidate what aspects of intermittency cause crabs to adopt indirect trajectories and, in particular, what the differences are between the two plume types that cause crabs in the Meandering plume to increase transverse movement while crabs in the Pulsed plume increase downstream movement?
- We know that crabs are responding to the frequency of stimuli above a threshold and there is some indication that crabs react to changes in concentration over time scales; however, we do not know the specific effects each of these plume properties (intermittency and concentration) have on blue crab tracking. Manipulative studies investigating the relative contribution of signal intermittency and signal concentration to the level of difficulty a particular plume presents to a tracking crab would help resolve these issues.
- Further quantitative analysis of vertical tracking behavior in blue crabs to understand the cues that affect vertical antennule placement during tracking.
- There appears to be a series of state dependent reactions that tracking blue crabs exhibit in response to seemingly similar stimuli. We do not currently know what these states are and therefore what causes, for example, the apparent "response" of crabs to accelerate upstream in the absence of antennule signals. I plan to analyze the current data set to determine if this state dependency is related to signal reception at the upstream legs, location within the plume, or changes in concentration.

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